REVIEW

Mechanisms for the Incorporation of Proteins in Membranes and Organelles

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Because of the high degree of organizational complexity of eucaryotic cells, it is clear that the implementation of their genetic programs must, in many cases, involve a complex sequence of cotranslational and posttranslational events that are necessary to transfer polypeptides from their sites of synthesis to their sites of function. It is difficult to envisage the existence of a single general mechanism that would ensure that all polypeptides released from ribosomes attain their correct subcellular destination. This is because there are, in a eucaryotic cell, at least as many possible destinations for a newly synthesized polypeptide as there are different compartments and membrane systems. Moreover, it is not likely that completed and fully folded polypeptides, with their charged and polar residues exposed to the aqueous environment, could freely traverse hydrophobic barriers constituted by phospholipid bilayers, which are the universal feature of all cell membranes (208). Instead, it may be expected that special mechanisms have evolved that direct polypeptides to specific membranes and, when necessary, assist them in their passage across the hydrophobic barriers. These mechanisms must involve specific receptors for structural features of the polypeptides and may entail conformational changes or even extensive structural modifications of the polypeptide, as well as the expenditure of energy.

In this paper we consider mechanisms for the transfer of newly synthesized polypeptides to their sites of function in different subcellular membranes and organelles, and discuss models in which specific features of the polypeptides serve as signals to direct them along selected subcellular pathways to their final destination. These signals may act during translation or after synthesis of the polypeptide is completed and may or may not be removed from the initial product of translation. Transient or permanent signals within polypeptides destined to membranes would also account for the final characteristic orientation of membrane proteins with respect to the phospholipid bilayer. Several other reviews discussing various aspects of this subject have recently appeared (15, 44, 53, 117a, 127, 243a).

Vectorial Discharge of Nascent Polypeptides: A Mechanism for Segregation of Proteins into the Lumen of the Endoplasmic Reticulum or Their Insertion into Membranes

Studies on the biosynthesis of secretory proteins first revealed the existence of a mechanism that effects the transfer of proteins across membranes concurrent with their synthesis (3, 16, 169, 170, 185, 188, 189). This mechanism, which is known as "vectorial discharge of nascent polypeptides" (146, 166, 185, 188), operates during the elongation phase of protein synthesis, i.e., before the polypeptide is fully folded, and involves the binding of ribosomes to specific sites on membranes of the rough endoplasmic reticulum (ER), sites which may be regarded as ribosome receptors (cf., 24, 115, 116, 117, 187).

The vectorial discharge of nascent polypeptides achieves its maximum expression in the extraordinarily well-developed ER of secretory cells of metazoa, where it ensures the transfer of large amounts of specific proteins into the ER lumen for subsequent transport into the Golgi apparatus, packaging into secretory granules, and eventual discharge from the cell (cf. 155). It has become apparent, however (cf. 155, 188), that proteins synthesized in membrane-bound ribosomes and released into the ER lumen are not all destined for secretion. Some such proteins may remain as permanent residents of the ER cisternae (116), while others may become associated with the luminal face of ER membranes as peripheral proteins, as appears to be the case with calsequestrin, a Ca^{++}-binding protein within the sarcoplasmic reticulum of muscle cells (79). Other proteins initially vectorially discharged into the ER lumen may be later diverted from the secretory pathway for segregation within membrane-bounded organelles (145). Thus, the lysosomal enzymes cathepsin D of spleen cells (55, 176) and β-glucuronidase of rat liver cells (164, 176) have been shown to be synthesized exclusively in bound polysomes. Since primary lysosomes appear to form in a special region of the endoplasmic membrane system that includes elements of the Golgi apparatus (GERL) (150), it is likely that other lysosomal enzymes are also initially cotranslationally discharged into the ER lumen. The synthesis of lysosomal enzymes on bound polysomes is also suggested by cytochemical studies, which demonstrated the presence of lysosomal hydrolases in the cisternal space of the ER of developing leukocytes (4).

It can easily be envisaged that variations of the basic mechanism of vectorial discharge that operates for the translocation of secretory proteins across the ER membranes can lead to the direct incorporation of other specific nascent polypeptides into the membrane, rather than to their release into the ER lumen. Indeed, several integral membrane proteins of the ER and plasma membrane are synthesized in bound polysomes and are

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first inserted into the rough ER membranes before being transferred to their ultimate destination. These include the Ca^{++}-ATPase of the sarcoplasmic reticulum (36, 79), cytochrome P-450 (5, 64, 147), epoxide hydrolase (76), and NADPH cytochrome P-450 reductase (75, 112, 114, 151), integral proteins of the ER that are present in both rough and smooth portions of this organelle. Integral membrane proteins of the plasma membrane that have been shown to be synthesized in bound polysomes include not only the well-studied envelope glycoproteins of vesicular stomatitis (VSV) (106, 107), Sindbis, and Semliki Forest viruses (20, 21, 67, 246) but also cellular plasma membrane proteins such as the hepatocyte 5'-nucleotidase (11), band 3, the major glycoprotein of the erythrocyte membrane (190), and the glycoprotein subunit of the Na^+,K^+-ATPase (201).

Although the process of vectorial discharge of nascent polypeptides was first recognized in the endoplasmic reticulum of eucaryotes, it is now clear that a similar process may operate in bacteria (8, 44, 94, 168, 198, 214, 243) and there are indications that a modified form of vectorial discharge also operates within semiautonomous organelles of eucaryotes such as mitochondria and chloroplasts. In these cases some of the organellar (33, 118, 200) or procaryotic ribosomes (214) have been reported to be bound, via their nascent polypeptides, to the inner organellar membranes. The products of such membrane-bound ribosomes could be transferred to the intermembrane or periplasmic spaces or be inserted into the inner or outer organellar or bacterial membranes. In the case of bacteria, it has been directly demonstrated that nascent polypeptides of enzymes destined to the periplasmic space span the membrane and can be labeled from the exoplasmic side by nonpenetrating reagents (212, 214).

Role of Cotranslational Insertion Signals in Determining the Initial Fate of a Nascent Polypeptide

A primary question concerning the synthesis of proteins on membrane-bound ribosomes stems from the specificity of this process: how is it determined that polysomes translating specific classes of messenger RNA molecules become associated with ER membranes, whereas others remain free in the cytoplasm and discharge their products into the cell sap? A second major question relates to the finer degree of specificity that is exerted in the operation of the vectorial discharge mechanism and, in particular, the features of the translation product that determine whether it is discharged into the cisternal lumen or is inserted into the ER membrane. It is clear that a full understanding of the process of vectorial discharge will require an elucidation of the detailed molecular interactions between ribosomes, membrane components, and specific regions of nascent polypeptides at the ribosome-membrane junction.

With respect to the first question, it is now widely acknowledged that the nascent polypeptide contains specific information that plays a major role in determining the site of translation. This was deduced from a series of observations that first demonstrated the role of the polypeptide in mediating the binding of ribosomes to ER membranes.

(*a*) It was first found that ribosomes are bound to the ER membranes via their large subunits that contain the nascent polypeptide chains (189). It was shown, however, that in spite of their topographical segregation, free and membrane-bound ribosomes are capable of exchanging subunits with each cycle of protein synthesis, a finding that strongly argued for their structural identity (22), which was subsequently demonstrated by electrophoretic analyses of ribosomal proteins (121, 130; c.f. 248). These observations suggested that structural features of the ribosome themselves do not determine the site in which they carry out translation.

(*b*) It was also demonstrated that, while ribosomes not containing nascent chains can be bound to specific receptors on the ER membranes, such ribosomes are removed from the membranes when microsomes are incubated in media of high salt concentration (2, 3, 22). On the other hand, ribosomes containing long nascent polypeptides can only be detached from microsomal membranes by treatment with puromycin, which releases the nascent polypeptide chain from the ribosome (2, 3), or by destructive methods that unfold the ribosomal subunits (189). Furthermore, it was shown that as the amino-terminal portion of the nascent chain emerges from the ribosome it enters into a relationship with the membrane that protects the extraribosomal segment of the nascent chain from attack by added proteases, unless detergents are added to dissolve the membranes (185).

It was therefore postulated (19) that information determining the association of specific polysomes with the ER membranes is contained in the amino-terminal segment of the nascent polypeptide chain, which acts as a signal determining the polysome-membrane interaction and in some way assists in the cotranslational translocation of the polypeptide across the microsomal membrane. This postulate is the essence of a "signal hypothesis" proposed to explain the polysome-membrane interaction (16, 17, 19), which, when presented by Blobel and Dobberstein (17), included speculative details on the mode of action of the signal in mediating the translocation. It was originally suggested that a channel under the ribosome exists in the membrane, or is created during translation, for the passage of the nascent polypeptide chain (19, 169, 187). Blobel and Dobberstein (16, 17) proposed that a role of the signal is to recruit membrane receptor proteins into the formation of a hydrophilic, multimeric, transmembrane tunnel, which is stabilized by a direct association of the receptor proteins with the large ribosomal subunit. The existence of a hydrophilic protein tunnel capped by the ribosome would ensure that the complete polypeptide chain, including the carboxy-terminal segment, which at polypeptide termination is still contained within the ribosome, crosses the membrane. The newly synthesized secretory polypeptide was therefore envisaged as diffusing unidirectionally into the ER lumen, with folding of the chain possibly serving to draw the entire molecule into the lumen (44, 119, 188).

Evidence supporting the essential postulate of the "signal hypothesis" was obtained when cell-free, messenger RNA–dependent synthesis of secretory polypeptides became possible. It was observed (16, 17, 47, 109, 138, 194, 223) that primary translation products synthesized in the absence of microsomal membranes (presecretory proteins) contain amino-terminal segments (referred to as signal segments), which are not present in the final secretory products or in products found within the microsomal lumen (prosecretory and secretory proteins). Indeed, Milstein et al. (138) produced an independent formulation of the signal hypothesis based on their observation that immunoglobulin light chains synthesized in vitro by microsomes, or in extracts that contained membranes, were processed by removal of an amino-terminal segment to a polypeptide with the same electrophoretic mobility as the mature light chain.

When translation occurs in association with microsomal membranes, the signal segment is removed before synthesis of the polypeptide is completed (16, 156, 195a) and, after polypeptide termination, the finished product is sequestered within the microsomal lumen, where it is inaccessible to exogenous proteases (17, 195b). Many signal segments of different presecretory proteins have been sequenced (see 112, 114, 218). Although their amino acid composition is variable, all signal sequences are characterized by a high proportion of hydrophobic amino acids. The fate of the cleaved signal segments is unknown, although it may be expected that these segments are removed from the membrane or degraded to ensure that they do not interfere with continuing translocation of nascent polypeptides. Nascent polypeptides must be longer than 30–40 amino acids, the length of the segments included within the large ribosomal subunits (18, 131) for their signals to emerge from the ribosomes and initiate insertion into the membrane. If, as suggested by its resistance to exogenous proteases, the peptidase that removes signal segments is located on the luminal side of the ER membrane (100, 237), the nascent polypeptide would have to attain a minimal length of 70–90 amino acids (30–40 plus 20 residues required to cross the membrane, in addition to the 18–30 residues of the signal segment) for the cleavage point to reach the enzyme and the signal segments to be removed. This estimate is substantiated by the steady-state distribution of intact and proteolytically processed nascent chains of secretory polypeptides within bound polysomes (16, 156). It is therefore possible that, for short secretory polypeptides, completion of synthesis may occur before processing has been quantitatively effected. This may explain the finding that small amounts of preproinsulin (158) and preparathyroid hormone (81) (109 and 115 residues, respectively) seem to escape the normal cleavage process and can be detected within cells. These findings could, perhaps, be also explained by an insufficiency of ribosome receptors in the ER membranes of these cells.

The existence of transient amino-terminal signal segments has been established for all eucaryotic secretory proteins so far examined, with the exception of ovalbumin (157), which is not proteolytically processed and appears to have a permanent insertion signal, probably located in the interior of the polypeptide (125, 126; but see 135). Several periplasmic proteins of *Escherichia coli*, which can be regarded as equivalent to secretory proteins, as well as outer membrane proteins of this bacterium, and which must also be transferred across the cytoplasmic membrane, have been shown to be synthesized with transient amino-terminal signals, largely hydrophobic and structurally similar to the signal segments of eucaryotic secretory proteins (93, 95, 143, 168, 221, 222). Direct evidence for a role of these segments in the translocation process has been provided by genetic experiments showing that mutations affecting specific residues within the signals prevent passage of the polypeptides across the membrane (6). In fact, DNA sequence analysis of mutant genes for altered polypeptides of the maltose binding protein of *E. coli* (9), which cannot be transferred to the periplasmic space, and for defective polypeptides of the lambda receptor protein (53), which do not reach the outer membrane and remain within the cytoplasm, has shown that all these mutations involved either substitutions in the signal sequence, from a single hydrophobic or uncharged amino acid to a charged one, or small deletions within the hydrophobic portion of the signals.

It should be noted, however, that cotranslational passage of specific periplasmic proteins through the cytoplasmic membrane of bacteria has been demonstrated directly only in a few instances (212–214, 233) and that there are at least two examples of proteins that contain amino-terminal signal sequences but appear to be incorporated into or transferred across the bacterial membrane posttranslationally (98, 99, 111). In one of these cases, that of the bacterial β-lactamase, in vivo studies have shown that normally a precursor of this periplasmic protein containing the signal sequence first appears in the cytoplasm, and that removal of the signal peptide is a subsequent step that occurs as the polypeptide is transferred across the membrane (111). Amber mutations of the β-lactamase have been obtained in which defective polypeptides, missing as few as 21 amino acid residues from the carboxyl terminus, are produced. Such polypeptides are not transferred across the membrane, although efficient cleavage of their signals seems to occur. These observations have been taken to indicate that a process fundamentally different from the cotranslational discharge that is envisioned to take place in the ER of eucaryotes ensures that the β-lactamase is transferred into the bacterial periplasmic space (111). It appears possible that, if this is the case, the lack of the carboxy-terminal segment in truncated polypeptides resulting from amber mutations may render the polypeptides insoluble in the intracellular milieu, thus preventing their subsequent passage through the membrane.

In this regard it is interesting to note that signal segments for eucaryotic secretory proteins synthesized by bacteria harboring recombinant plasmids (61, 228) have been found to be capable of mediating secretion of the polypeptide through the bacterial membrane. Thus, preproinsulin containing most of the natural eucaryotic signal sequence is effectively processed by *E. coli* to the mature proinsulin that is secreted from the cell (227). It has recently been found, however, that the amino-terminal segment of the β-lactamase precursor, when fused to the eucaryotic proinsulin polypeptide, is also capable of determining proinsulin secretion from the bacterium (227). These results are difficult to interpret without confronting the disturbing possibility that in bacteria proinsulin may follow more than one secretory pathway, depending on the signal to which it is attached. Alternatively, it is possible that the same signal sequence that leads to cotranslational discharge in eucaryotic cells is utilized posttranslationally in bacteria. Since β-lactamase polypeptides lacking a carboxy-terminal segment could not be exported (111), while proinsulin fused to the lactamase signal was efficiently secreted, one must also conclude that a carboxy-terminal sequence that is normally transferred cotranslationally across the eucaryotic membrane can substitute for a lactamase sequence that in the bacterium appears to be functionally important in a posttranslational translocation process.

Cotranslational Transfer of Polypeptides through ER Membranes

The findings discussed in the previous section are consistent with the notion that signal segments of nascent secretory polypeptides play an essential role in initiating the passage of the polypeptides across membranes, although the mechanistic details of this process remain to be elucidated. The apparent unrestricted capacity of microsomal membranes from dog pancreas (16) HeLa cells (112), ascites tumor cells (138, 225), and hen oviduct (229) to effect, in vitro, the vectorial transfer, removal of signal peptides, and cotranslational glycosylation of a wide variety of presecretory polypeptides from different species and cell types indicates that different signal segments

are functionally equivalent and probably participate in a single translocation mechanism that has been conserved during evolution. Because comparison of the amino acid sequences of signal segments of numerous presecretory proteins fails to show a strong sequence homology, it has been concluded (94, 101, 114, 218) that conformational features of the signals, rather than specific primary sequence information, are the functionally important elements that may be recognized by specific receptors in ER membranes. Different signal segments resemble each other in that they contain middle regions of hydrophobicity, and frequently one or two charged residues are present near the amino-terminal end. It has also been pointed out that amino acids with the smallest side chains are frequently found near the cleavage point of the signal segments (94).

The hydrophobic character of the middle region of the signal segment seems ideally suited for initiating an interaction with the lipophilic interior of the membrane. Positive charges at the amino terminus and in adjacent lysines and arginines (when present) or negatively charged amino acids that are also sometimes found in signal sequences near the amino-terminal end may serve to facilitate the initial association of the nascent polypeptide with the membrane through ionic interactions with the polar head groups of membrane phospholipids (94). In fact, because of its charge, the amino-terminal end of the signal segment may remain exposed on the cytoplasmic side of the membrane, so that during vectorial discharge the nascent polypeptide would acquire a looped disposition that would be maintained until the signal segment is removed by proteolysis (94, 114, 218). This loop model differs in several important aspects from a simpler model used in earlier work in this area (16, 17, 185, 186), in which the amino terminus of the nascent chain was depicted as being inserted directly into the membrane, ultimately reaching the luminal side. If the charges present at the amino terminus of the nascent chain play an important role in determining the looped configuration, then it could be envisaged that when the signal is not removed by cotranslational cleavage, as is the case with ovalbumin, the absence of charges could facilitate transfer of the amino terminus across the membrane. An important feature of the looped configuration is that it could be attained even if insertion signals were not located in the amino-terminal region of the polypeptide, as may be the case for ovalbumin (125) and for certain membrane proteins in which the amino-terminal region remains exposed on the cytoplasmic side of the membrane (see below).

Despite the critical role of signal segments in initiating vectorial discharge, it should be emphasized that a direct interaction of the ribosomes with binding sites in the membrane, which has long been recognized in the ER but has not been demonstrated in procaryotes (210, 211), may also play an important role in polypeptide translocation. Microsomal membranes have been shown to contain specific receptors for ribosomes capable of interacting directly with the large ribosomal subunits in media of physiological ionic strength, even in the absence of nascent polypeptides (24; for review, see 188). Moreover, the number of ribosome binding sites found in rat liver microsomal membranes stripped of ribosomes was much higher than in smooth microsomes. The functional capacity of microsomal membranes stripped of ribosomes to rebind ribosomes and to effect the cotranslational discharge of secretory polypeptides was shown to depend on the integrity of proteins exposed on the surface of the microsomal membrane (24). The identification of the specific microsomal membrane proteins involved in ribosome binding and/or recognition and processing of the signal peptides is an important area of current investigation. Comparison of polypeptide patterns of rough and smooth microsomal membranes from rat liver (115, 117) and other sources (114, 117) has revealed the existence in rough microsomes of two major membrane polypeptides that have been designated ribophorins I and II and have molecular weights of ~65,000 and ~63,000. These proteins are absent from membranes derived from the smooth ER and their content in different rough microsomal fractions was found to be stoichiometrically related to the number of ribosomes (117). Differential extraction of microsomes with high concentrations of certain nonionic detergents was shown to leave membrane remnants in which the association between ribophorins and ribosomes is maintained despite the removal of most other membrane proteins (113, 117). Moreover, when a redistribution of ribosomes on the microsomal surface was caused by treatment of rough microsomes with low concentrations of neutral detergents, which lead to the formation of ribosome aggregates in invaginated areas of the microsomal membrane, the ribophorins seemed to redistribute together with the ribosomes. This was deduced from the finding that when, at higher detergent concentrations, the microsomes carrying ribosomal aggregates fragmented into small rough inverted vesicles and smooth vesicles completely devoid of ribosomes (113), the ribophorins were the only membrane proteins found exclusively in the ribosome-containing subfraction. A close proximity and a specific association between the ribophorins and the ribosomes in native microsomes was also demonstrated using reversible crosslinking reagents that allowed the recovery of these proteins with sedimentable ribosomes, after other membrane proteins were solubilized with the anionic detergent sodium deoxycholate (Na DOC) (117). These findings led to the suggestion that ribophorins play a role in ribosome binding and possibly other aspects of the translocation mechanism.

It should be noted, however, that the translocation and processing activity of rough microsomal membrane fractions in in vitro experiments is always much lower than expected from the ribosome and ribophorin content of the native membranes measured by gel electrophoresis. It is not clear that stripping of ribosomes improves the translocation capacity of the membranes and it is possible that only sites that before cell fractionation were naturally unoccupied by ribosomes and nascent chains are capable of carrying out a complete vectorial discharge in in vitro translocation experiments with stripped or intact microsomal membranes. Ribosome binding experiments show that these available sites may be a small fraction of the total number of sites in rough microsomes, but they may be the only ones present in the membranes found in smooth microsomal fractions. This could explain the recent report that rough and smooth microsomal fractions (13) may have comparable in vitro translocation and processing activities. The latter finding may have also resulted from differential losses of translocation activity during the preparation of microsomes.

Other evidence for the role of specific microsomal proteins in the vectorial discharge of nascent polypeptides has recently been presented. In particular, polypeptides that can be released by high salt from normal microsomes (238) (probably after they have been cleaved by endogenous proteases during preparation of the microsomes) or that can be removed after mild digestion with exogenous enzymes have been shown to be able to restore the translocation capacity of trypsinized or depleted microsomes (237). The active polypeptide released by protease

digestion was found to contain a sulfhydryl group that is necessary for restoring the translocation function (102, 136, 137).

A tight binding of the ribosome to its receptor on the ER membrane may provide an important energy contribution to the process of polypeptide translocation (236). An extreme alternative to mechanisms requiring a protein tunnel for the cotranslational passage of the polypeptide through the membrane is one in which the polypeptide is directly inserted into the phospholipid bilayer using the energy of protein synthesis. It may be envisaged that once insertion of the nascent polypeptide into the membrane is initiated by the hydrophobic region of the signal sequence, the association of the ribosome with its receptor site would serve to ensure that amino acid residues that follow the signal are inserted into the lipophilic membrane as soon as they emerge from the ribosome. As elongation proceeds and the nascent polypeptide chain reaches the luminal side of the membrane, a steady state would be achieved during which the entry of subsequent amino acid residues into the cytoplasmic side of the membrane is compensated by the exit of other residues from the luminal face. Finally, once a sufficiently long segment of the polypeptide has emerged into the aqueous environment of the cisternal space, its folding, too, could assist in drawing the remaining portion of the chain, including the carboxy-terminal end, through the membrane (cf. 44). Indeed, in the absence of a tunnel or an active energy-consuming translocator within the membrane, folding of the polypeptide at the luminal side of the membrane could be the only motive force used to transfer the carboxy-terminal segment after termination of polypeptide synthesis has occurred (188). If this is the case, one would expect that the amino acid sequences of carboxy-terminal regions of secretory proteins have evolved to facilitate their passage across the membrane in the absence of the protein synthetic motive force.

Recently, an analysis has been presented (236) of the energy requirements that must be met to effect the direct cotranslational transfer of proteins of known primary sequence across a membrane. It was concluded that for several secretory and bacterial outer membrane polypeptides, the energy provided by the association of the ribosome with its binding site (28,000 cal/mol, calculated from an affinity constant of 10^{-7} M [24]) could, without the need of a receptor for the signal peptide or the assembly of a channel within the membrane, suffice to ensure the cotranslational translocation of these polypeptides across the membrane. In this view, secretory polypeptides would have, in the course of evolution, acquired appropriate sequences for their direct cotranslational transfer across the phospholipid bilayer. Furthermore, the main function of the signal segment would be to initiate the insertion of the polypeptide into the ER membrane via a simple hydrophobic interaction with the interior of the membrane. It should be noted, however, that evidence has been presented suggesting the existence within microsomal membranes of specific receptors for signal sequences. Thus, a segment of ovalbumin containing its putative signal sequence when added at high concentrations was able to block the cotranslational translocation and processing of other secretory proteins (125). More recently, it has been reported that in-vitro-synthesized preproinsulin, but not proinsulin, binds to dog pancreas rough microsomes and, when present in saturating amounts, abolishes the capacity of these membranes to effect the subsequent cotranslational transfer of nascent polypeptides (166).

Although the interaction of the signal with the membrane is thought to be necessary to initiate transfer of the nascent polypeptide across the membrane, it is clear that covalent attachment of the signal to the rest of the polypeptide is not required for continuing vectorial transfer. This can be inferred from the fact that in most secretory proteins the signal is cleaved from the body of the polypeptide much before other portions of the polypeptide, which must be transferred across the membrane, have been synthesized (16, 156). Because the primary role of the signal segment is to determine insertion of the nascent polypeptide into the ER membrane, we designate it as a "signal for cotranslational insertion" (or "cotranslational insertion signal"). In view of the role of the cotranslational insertion signal, and given its hydrophobic character, one might expect that cleavage of the polypeptide and loss of the signal are necessary for the release of the protein into the ER lumen. The case of ovalbumin shows, however, that cleavage is not an absolute requirement to release the polypeptide from the membrane or to dislodge the signal from its membrane receptor. Insertion of this protein has been reported to occur via a signal that is not removed by cleavage (157), but is functionally similar to the transient cotranslational insertion signals found in other secretory proteins (125, 126). This signal may be located in the interior of the polypeptide (125) but its location and permanence do not prevent discharge of the completed translation product into the microsomal lumen.

It might be argued, on the basis of the observations with most secretory proteins, that, although removed from the rest of the polypeptide by an endoprotease, the cotranslational insertion signal must remain associated with its receptor throughout vectorial discharge to, perhaps, maintain an open passageway for the nascent chain. If this is the case and if the insertion signal for ovalbumin is indeed an interior part of the mature protein (125), then the peptide segment that serves as a signal would have to be transferred across the membrane after termination of polypeptide synthesis and after all other segments of the molecule have been translocated into the ER lumen.

The Disposition of a Protein in a Membrane as a Result of the Mechanism of its Cotranslational Insertion

The orientation of an integral membrane protein with respect to the phospholipid bilayer is likely to offer clues as to the mechanism of its insertion into the membrane. Many cellular and viral transmembrane proteins, such as the erythrocyte glycophorin (27, 231, 40), heavy chains of membrane-associated IgM (234, 108) and the histocompatibility antigens (249, 160), as well as the envelope glycoprotein (G protein) of VSV (106) have their amino-terminal ends exposed on the luminal or extracellular face of membranes and the carboxy-terminal portions on the cytoplasmic side. It may be postulated that all proteins of this transmembrane disposition are synthesized in membrane-bound ribosomes and that their orientation results from cotranslational insertion of the polypeptides into the membrane by a process akin to an interrupted vectorial discharge (Fig. 1). It would then be expected that the nascent polypeptides contain amino-terminal cotranslational insertion signals analogous to those in secretory proteins, which initiate the insertion, as well as signals that may be referred to as "halt" or "stop" transfer signals (5, 14, 36, 124) that cause an interruption of the vectorial discharge across the membrane, leading

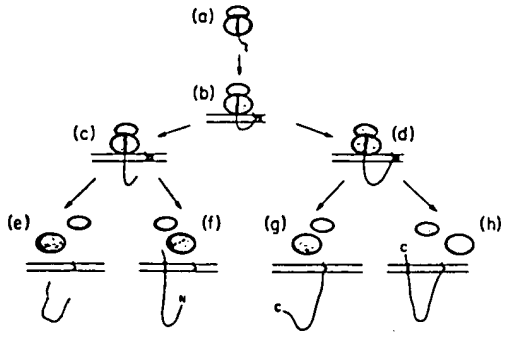


FIGURE 1 Models describing the role of amino-terminal cotranslational insertion signals in the insertion of integral membrane proteins into the ER membrane. In these models, membrane protein biosynthesis is depicted as an interrupted vectorial discharge process. The initial stages of cotranslational insertion of secretory and integral membrane polypeptides are similar. An amino-terminal cotranslational insertion signal (>) emerges from the ribosome (*a*), and interacts with a receptor (◗) within the ER membrane, leading to ribosome attachment (*b*), which facilitates passage of distal regions of the polypeptide through the membrane. The amino-terminal cotranslational insertion signal may be a transient (*c*) or permanent feature (*d*) of the nascent polypeptide. Cotranslational cleavage by a membrane-associated signal peptidase (*c*) ensures release of the polypeptide into the ER lumen (*e*) unless vectorial discharge is interrupted by a halt transfer signal (I) that prevents passage of the carboxy-terminal portion of the polypeptide through the membrane (*f*). In the absence of a halt transfer signal, an uncleaved amino-terminal cotranslational insertion signal may serve to maintain the permanent association with the membrane of proteins that are otherwise fully exposed on the luminal side of the membrane (*g*). If the permanent amino-terminal cotranslational insertion signal is followed by a halt transfer signal, both termini of the protein remain on the cytoplasmic side while the rest of the chain forms a loop on the luminal aspect of the membrane (*h*). *C*, carboxy-terminal portion.

to retention of the protein in the phospholipid bilayer (Fig. 1*f*). For membrane proteins, however, cleavage of the amino-terminal signal may not be an obligatory step of the insertion process, particularly if that portion of the polypeptide remains associated with the membrane in the mature product (Fig. 1 *g* and *h*). For example, in bacteria it has been shown (122) that a mutational alteration in the signal sequence of the *E. coli* outer membrane lipoprotein, which prevents cleavage of the signal sequence from the rest of the polypeptide, does not block transfer of this protein to its destination.

In the polypeptides listed above, halt transfer signals (see below) would be represented by membrane-embedded hydrophobic segments followed by charged residues that are located very near the carboxy-terminal ends of the molecules. In these cases, therefore, only a short polypeptide segment remains exposed on the cytoplasmic face of the membrane after termination of polypeptide growth (Fig. 1*f*). However, there is no logical reason to postulate that halt transfer signals must always be located so close to the carboxyl terminus of the protein. In fact, integral membrane proteins synthesized in bound polysomes may have large functional segments exposed on the cytoplasmic side of the membrane. In these cases, translation would continue after the halt transfer signal is inserted into the membrane. When, after the halt transfer signal, a polypeptide segment longer than the region of ~40 amino acids that can be contained within the ribosome is synthesized, dislodging of the ribosome from its binding site could occur, and polypeptide synthesis would then continue in the cytoplasm. Indeed, dislodging of the ribosome from its binding site could, at least in part, be induced by a high concentration of charged amino acid residues on the cytoplasmic, carboxy-terminal side of the halt transfer signal. Alternatively, ribosome detachment may simply result from steric hindrance due to accumulation under the ribosome of polypeptide segments that cannot penetrate the membrane.

The existence of a transient amino-terminal signal for the cotranslational insertion of the envelope G protein of VSV has been demonstrated by the finding that the translation product of the G mRNA obtained in the absence of membranes (96, 126) contains an amino-terminal segment that is similar to that found in presecretory proteins, which is absent from the polypeptide found in microsomal membranes of infected cells. This segment is presumed to initiate association of the nascent polypeptide with the ER membrane, an event that, for this protein, has been shown to occur before the polypeptide reaches a length of 80 amino acid residues (182). The signal segment of the VSV G protein is removed during translation (124) and therefore the initial phase of the insertion of this polypeptide into the membrane is similar to that which initiates vectorial discharge of presecretory proteins into the ER lumen. The fundamental mechanistic similarity between these two processes (Fig. 1 *e* and *f*) is made apparent by the fact that, in in vitro reconstruction experiments with exogenous membranes, polysomes synthesizing the G protein of VSV can compete with polysomes synthesizing a secretory polypeptide and prevent its processing and vectorial discharge (124). The essential difference between them is that the membrane protein remains associated with the ER membrane after cleavage of the cotranslational insertion signal and achieves a transmembrane disposition in the ER that is maintained as the polypeptide is transferred to the plasma membrane and is incorporated into viruses by the budding process (106, 107).

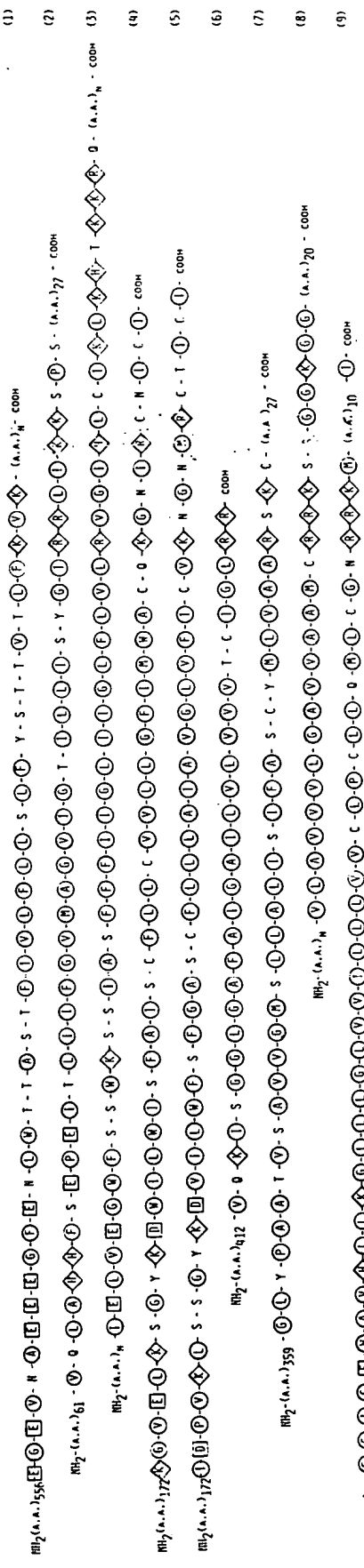
Halt or Stop Transfer Signals

In discussing the cotranslational insertion of transmembrane polypeptides, it is worthwhile to consider mechanisms by which halt transfer signals could interrupt the process of cotranslational passage across the membrane. To accommodate a translocation model that involves a hydrophilic multimeric protein tunnel through the membrane, Blobel and his associates (16) have suggested that halt transfer signals function by triggering disassembly of this tunnel. In this case it might be expected that the polypeptide then undergoes some reorientation to achieve its most stable configuration in the membrane. On the other hand, halt transfer signals may simply be represented by the same structural elements of the polypeptides that serve to establish a stable interaction with the phospholipid bilayer and to position the mature membrane protein in the membrane. It is clear, however, that the hydrophobic character of such segments would not be sufficient to prevent passage of distal regions of the nascent polypeptide through the membrane. In fact, a region of hydrophobicity characterizes the amino-terminal cotranslational insertion signals of secretory proteins, which serve as temporary anchors of nascent polypeptide chains to the membrane but, as an essential function, facilitate rather than halt translocation across the ER membrane. Furthermore, it has been demonstrated that the amino terminal portions of proteins of enveloped viruses such as the F1 (fusion) proteins of SV5 (simian virus) (32), and Sendai virus (70),

contain hydrophobic segments that during synthesis on bound polysomes must be transferred cotranslationally across the ER membrane.

An examination of the sequences of membrane-associated segments of several membrane proteins (Scheme 1) is consistent with the notion that halt transfer signals include, in addition to a hydrophobic segment, an adjacent group of highly charged amino acid residues immediately following the carboxy-terminal end of the hydrophobic region. These charged residues would not be expected to penetrate the phospholipid bilayer and therefore would remain on the cytoplasmic side. The amino acid sequence of the membrane-associated segment of the G protein has been deduced from the DNA sequence of a cDNA clone, and confirmed by partial amino acid sequence analysis of a fragment that remains within the viral membrane after protease digestion (175). Twenty hydrophobic amino acid residues appear to be embedded in the membrane, while the carboxy-terminal distal segment of 30 amino acids exposed on the cytoplasm contains a high proportion of basic residues. This arrangement is similar to that in glycophorin, the major glycoprotein of the erythrocyte membrane (40, 231), which contains a transmembrane segment of 23 hydrophobic residues followed by a carboxy-terminal, highly charged, but not strongly basic, cytoplasmic segment (36 amino acid residues). The hemagglutinin of influenza virus represents another example of a viral protein anchored to the membrane by a polypeptide segment near its carboxy-terminal end (52). This protein consists of two disulfide-linked polypeptides, HA-1 and HA-2, which are derived by posttranslational cleavage from a precursor that is the translation product of a single mRNA molecule, but only HA-2, which represents the carboxy-terminal portion of the precursor, is directly anchored in the membrane. A transient amino-terminal signal segment initiates cotranslational insertion of the HA-1 portion of the precursor into the membrane and vectorial discharge is presumably interrupted during the transfer of the HA-2 segment by a signal near its carboxy-terminal end. The amino acid sequence of HA-2 polypeptides of several strains of influenza and fowl plague virus have been deduced from the DNA sequence of cDNA clones (139). Like the VSV G protein, these polypeptides also contain putative halt transfer signals represented by hydrophobic segments (24 amino acids long), followed by short (11 amino acids) carboxy-terminal hydrophilic regions, which are thought to be exposed in the cytoplasm. It is worth pointing out that all the putative halt transfer signals listed in Table I are located very near the carboxy-terminal end of the molecules. In fact, since a carboxy-terminal segment of the nascent chain of ~40 amino acids is contained within the ribosome, for some of the proteins listed termination of peptide synthesis must occur before the hydrophobic segment positions the polypeptide in the membrane. A halt transfer signal, however, need not always be located near a termination codon. This is the case, for example, with the Semliki Forest virus E2 envelope glycoprotein, which is inserted into the membrane as part of a nascent polyprotein. In addition, bacterial chimeric proteins, described in detail below, have been constructed through the fusion of genes for the lambda receptor and β-galactosidase, which are inserted normally into the membrane (54) and contain a large portion of the β-galactosidase exposed on the cytoplasmic side following the carboxy-terminal end of a putative halt transfer signal.

It should be noted that, in addition to halt transfer signals, other features of membrane polypeptides that may be acquired



SCHEME 1 Putative halt transfer signals for several plasma membrane proteins. In all cases a hydrophobic segment varying in length from 20 to >32 is followed by a group of positively charged amino acids that is thought to be exposed on the cytoplasmic side of the membrane. In the case of glycophorin, the polypeptide was sequenced and the location of the hydrophobic segment within the membrane was determined directly (cf. reference 231). In all other cases, the amino acid sequences were derived from DNA sequence data, although in the case of VSV G protein and of the histocompatibility antigen amino acid, sequence data are also available. (1) Immunoglobulin μ_m-chain (174); (2) glycophorin A (231); (3) VSV G (175); (4) A/Victoria/3 75 (H3N2) influenza A hemagglutinin (70, 139); (5) A/Aichi/2/68 (H3 subtype) influenza A hemagglutinin (70, 139); (6) Semliki Forest virus E1 protein (66); (7) Semliki Forest virus E2 protein (66); (8) histocompatibility antigen HLA-B7 (162); (9) glycoprotein of avian sarcoma virus (41). Amino acids were designated as basic (◇), acidic (□), nonpolar hydrophobic (○) or neutral (not specifically marked).

by posttranslational modifications may also contribute to stabilize their association with the phospholipid bilayer. Such may be the role of fatty acid moieties that have been shown to be linked covalently to viral envelope glycoproteins (197).

Secretory polypeptides must, of course, be free of sequences that could halt their transfer through the membrane and thus prevent their discharge into the ER lumen. Analysis of the energy requirements for transferring several secretory proteins of known amino acid sequence across hydrophobic barriers suggests that this is indeed the case (236). The notion that sequences of secretory proteins have evolved to eliminate potential halt transfer signals is reinforced by studies with hybrid proteins created by genetic manipulation of *E. coli*. Through gene fusion experiments, hybrid proteins were produced in which portions (200–300 amino acids) of the periplasmic maltose binding protein containing its amino-terminal signal sequence were linked to an enzymatically active portion of the cytoplasmic protein β-galactosidase. In this case, the hybrid protein was not discharged into the periplasmic space, but appeared to be retained (7) in the cytoplasmic membrane. This could result from interruption of the vectorial discharge by a pseudo halt transfer signal sequence present in β-galactosidase, a protein that normally does not traverse a membrane.

It should be noted, however, that the insertion of large amounts of these polypeptides into the inner bacterial membrane, after induction of the maltose operon, had lethal consequences for the cell, appearing to jam the vectorial discharge apparatus and to prevent other proteins that accumulated as precursors in the cytoplasm from being transferred across the membrane. A similar situation was obtained when chimeric proteins were constructed that contained approximately the amino-terminal half, including the cotranslational insertion signal segment, of the outer membrane lambda receptor protein linked to β-galactosidase (199). On the other hand, if the amino-terminal segment from the lambda receptor protein contained in the chimeric protein was just slightly longer, the insertion was no longer lethal, the export machinery was not jammed, and the chimeric protein inserted into the inner membrane was apparently transferred efficiently to the outer membrane of the bacterium (53).

Notably, gene fusions yielding chimeric proteins in which the periplasmic maltose binding polypeptide was linked with β-galactosidase have been found to be always lethal after induction of the maltose operon even when almost the entire maltose binding protein was included in the amino-terminal portion of the chimeric molecule (7). These observations indicate that, after complete passage of the maltose binding protein segment, direct cotranslational insertion of the pseudo halt transfer signal of β-galactosidase into the membrane incapacitates the normal mechanism for vectorial transfer of polypeptides through the membrane and is therefore deleterious to the cells. The results with chimeric proteins containing sequences of the lambda receptor indicate, however, that a specific peptide segment located approximately in the middle of this membrane polypeptide prevents the deleterious insertion of β-galactosidase, presumably by halting transfer of distal regions of the chimeric molecule into the membrane (53). The term "dissociation sequence" was used to designate this specific segment of the lambda receptor, which, it was proposed, leads to detachment of the ribosome from the membrane, allowing for reutilization of the vectorial transfer apparatus (53). It can be presumed that the dissociation sequence, which is absent from the secreted maltose binding protein, also ensures retention of the lambda receptor in the membrane and therefore serves as a halt transfer signal. From the analysis of these experiments, the concept emerges that halt transfer signals should have specific properties that enable them not only to interrupt the vectorial discharge but also to allow for reutilization of the vectorial transfer apparatus, presumably the ribosome binding site and its associated structures. It is worth noting that, if placed sufficiently near the insertion signal, pseudo halt transfer features of cytoplasmic polypeptides may lead to rejection of the hybrid protein nascent chain into the cytoplasm rather than to its stable incorporation into the membrane and its ensuing deleterious consequences for the cell. Such appears to be the case with a chimeric protein containing β-galactosidase and only the first 39 amino acids of the lambda receptor protein including its entire 24 amino acid signal sequence (140).

As mentioned previously, biosynthetic mechanisms similar to those utilized for the insertion of viral envelope glycoproteins into the ER membrane would be expected to operate during the synthesis of cellular G proteins with the same transmembrane dispositions. In fact, studies on the in vitro synthesis of the mouse H-2D (49) and human HLA (161) histocompatibility antigen show that these polypeptides are cotranslationally inserted into ER membranes with what appears to be the correct transmembrane disposition. The H2-D polypeptide synthesized in vitro (49) in the absence of membranes was found to be 1,000–2,000 daltons larger than the nonglycosylated form of the mature protein synthesized in tunicamycin-treated cells. From these observations it was inferred that a transient cotranslational insertion signal is contained in the nascent primary translation product. It should be noted that the heavy chain of the histocompatibility antigen is largely exposed on the extracellular surface of the cell (202), where it is associated noncovalently with β_2-microglobulin (144). The latter is a low molecular weight polypeptide that is also found in circulating plasma. This polypeptide, which may be regarded as a peripheral membrane protein, is synthesized as a typical secretory protein with a transient amino-terminal signal for cotranslational insertion (49, 123) and its noncovalent attachment to the heavy chain appears to occur immediately after its discharge into the ER lumen (49).

The capacity of B lymphocytes to produce, at different stages of development, plasma membrane–associated and secretory forms of IgM molecules has provided another interesting system for investigating mechanisms that determine whether a polypeptide synthesized on bound polysomes is discharged into the ER lumen, to be ultimately secreted from the cells, or is inserted into the ER membrane with a transmembrane disposition for possible subsequent transfer to the plasma membrane. Recent studies have indicated that membrane-associated and secretory IgM contain μ-chains that differ in peptide segments located at their extreme carboxy-terminal ends (108, 206, 234). Comparison of sequences of cDNA clones obtained from the mRNA of a single myeloma tumor revealed the existence of two types of messenger RNA for μ chains, which differed only in nucleotide sequences at their 3′-terminal ends (174). One form encoded a carboxy-terminal region of 20 amino acids that is characteristic of the secreted polypeptide (μ_s). This contains an asparagine residue to which oligosaccharide is probably linked and a cysteine residue that, within the pentameric secreted IgM, forms a disulfide bond with another polypeptide chain (J) that is a component of secreted IgM. The other type of μ-chain messenger RNA lacked the nucleotide sequence

coding for the "secretory" segment but encoded instead a carboxy-terminal segment of 41 amino acid residues that, in the membrane-associated IgM (μ_m), is likely to function in anchoring the immunoglobulin to the membrane (174). This segment contains a group of 26 uncharged amino acids (with a hydrophobic core of eleven residues) that is flanked at its amino-terminal side by a negatively charged segment of 12 amino acids (including six glutamic acid residues) and at its carboxy-terminal side by a tripeptide containing two lysines.

The carboxy-terminal segment of the membrane form of μ-chain most probably serves as a halt transfer signal that during synthesis in bound polysomes interrupts the cotranslational discharge of the μ_m-chain through the membrane. The extreme carboxy-terminal location of a stop transfer signal in the μ-chain would ensure that after transfer to the plasma membrane all the rest of the IgM molecule is exposed on the surface of the cell and is accessible for interaction with antigen.

It is most interesting to note that analysis of genomic clones containing the μ-chain gene indicates that the two forms of μ-chain mRNA are derived from a single genomic arrangement of coding (exons) and noncoding (introns) DNA regions, which are utilized differentially to yield mRNA coding for the two forms of μ-chains (51). In one case, immediately after transcription of the secretory gene segment, cleavage and polyadenylation of the nascent RNA transcript would take place, yielding, after removal of all proximal introns, the μ_s mRNA. In the other case, transcription would continue without cleavage and poly(A) addition to include, in the transcript, exons coding for the membrane segment and two additional introns. Processing of this RNA would eliminate the secretory segment, which is now treated as part of an intronic region, to yield the μ_m mRNA. This phenomenon, in which different forms of messenger RNA for related proteins are derived from a single "gene," is somewhat analogous to what occurs in polyoma virus–transformed cells: three different forms of tumor antigen, with identical amino-terminal sequences (92, 210) but substantially different carboxy-terminal regions and different subcellular localizations (nuclear, plasma membrane, and cytoplasmic) (97), apparently originate from a single RNA precursor (215, 62). In this case, nature seems to have already performed an experiment that demonstrates that the specific subcellular localization of each of these polypeptides, which most likely are synthesized in free polysomes, cannot be determined by the amino-terminal sequences that are common to all of them.

Permanent and Interior Insertion Signals in Membrane Proteins

Our studies on the synthesis of the ER membrane protein cytochrome P-450, which is synthesized exclusively in membrane-bound ribosomes (5), indicate that signals for the cotranslational insertion of membrane polypeptides need not always be transient and therefore may remain in the mature protein. The amino-terminal sequence of the primary translation product of the phenobarbital-induced form of cytochrome P-450, synthesized in vitro using an mRNA template in the absence of added microsomal membranes, was found to correspond to that of the mature product. This is likely to be also true for other forms of cytochrome P-450 (25) and for the microsomal epoxide hydratase (50, 76). Amino-terminal segments of these proteins, which are rich in hydrophobic amino acids and resemble the signal sequences present in presecretory proteins, are likely to function as signals for cotranslational insertion (Fig. 1 *a*, *b*, and *d*). Were the amino-terminal putative cotranslational insertion signal of cytochrome P-450 the only signal present, then one might have expected, by analogy with secretory proteins, that the entire molecule would pass through the membrane, perhaps remaining attached to it on the luminal side by the permanent insertion segment (Fig. 1 *g*). Since, however, a portion of the cytochrome P-450 molecule is known to be exposed on the cytoplasmic side of the microsomal membrane (46, 134, 173, 230, 242), one must postulate that the polypeptide also contains a halt transfer signal that interrupts cotranslational transfer through the membrane, leaving a portion of the molecule exposed on the cytoplasmic surface (Fig. 1 *h*). It should be noted that the hydrophobic amino-terminal portion of cytochrome P-450 is considerably longer than the usual cotranslational insertion signals of secretory proteins (our own unpublished observations) and, therefore, in this case, interruption of the vectorial discharge may occur soon after insertion.

The absence of a halt transfer signal, however, and the presence of an uncleaved amino-terminal cotranslational insertion signal that remains membrane associated may explain the orientation of some plasma membrane proteins such as the sucrase isomaltase glycoprotein of the microvillar membranes of intestinal cells. This enzyme, which consists of two polypeptide subunits derived from a single precursor by posttranslational processing (88), appears to be anchored to the membrane only through a hydrophobic region near the amino-terminal end of the isomaltase subunit and, therefore, is almost completely exposed on the outer surface of intestinal cells (30, 59).

The orientation of certain membrane polypeptides with respect to the phospholipid bilayer suggests that signals for cotranslational insertion need not always be at the amino-terminal end of the polypeptide. If a polypeptide contains an internal cotranslational insertion signal (Fig. 2), attachment of the ribosome to the membrane would occur late in translation, but the initial disposition of the nascent chain within the membrane (Fig. 2 *b*) would still be analogous to that envisioned in the loop model for proteins containing amino-terminal cotranslational insertion signals. Interior insertion signals would, of course, also lead to the cotranslational passage of distal portions of the polypeptide through the membrane. In this case, however, the final disposition of the protein would be as indicated in Fig. 2 *c*, with an amino-terminal segment exposed on the cytoplasmic side of the membrane and the carboxy-terminal portion on the luminal side. An interior insertion signal of this type may account for the fact that the erythrocyte plasma membrane protein band 3, which is synthesized in membrane-bound ribosomes (190), has, in its final disposition, the amino-terminal portion exposed on the cytoplasmic face of the membrane (cf. 216). If, in addition to the interior cotranslational insertion signal, a polypeptide also contains a subsequent halt transfer signal (Fig. 2 *d*), then the final disposition of the protein would be as depicted in Fig. 2 *e* with both amino- and carboxy-terminal ends exposed on the cytoplasmic side of the membrane with an interior loop remaining on the luminal side (Fig. 2 *e*). Different dispositions of transmembrane proteins, including several crossings of the phospholipid bilayer, can be accounted for by a generalization of this model, if one postulates that proteins synthesized in membrane-bound ribosomes may contain multiple cotranslational insertion and halt transfer signals within a single polypeptide (Fig. 2). It should be noted that the location of the carboxy-terminal end of a transmembrane protein on the luminal or the cytoplasmic face of the membrane would depend

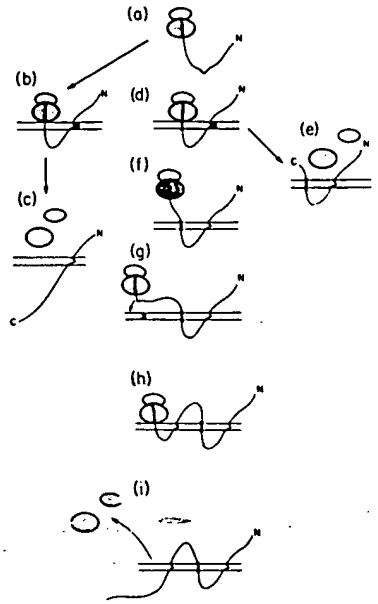


FIGURE 2 Models explaining the transmembrane dispositions of polypeptides as resulting from the presence of one or more permanent interior cotranslational (*a*) insertion and halt transfer signals. An interior cotranslational insertion signal leads to association of the ribosome with the membrane late in translation (*b*), leaving an amino-terminal portion (*N*) of the polypeptide exposed on the cytoplasmic side. In the absence of a halt transfer signal, the entire carboxy-terminal portion (*C*) of the polypeptide traverses the membrane and becomes exposed on the luminal side (*b* and *c*). In this case, the transmembrane orientation of the polypeptide is the reverse of that shown in Fig. 1, and the permanent association of the polypeptide within the membrane is maintained through the original insertion segment. The presence of a halt transfer signal (*d–f*) determines that growth of the nascent polypeptide continues on the cytoplasmic side of the membrane where it may be completed with (*f*) or without (*e*) ribosome detachment from the membrane before termination. As pointed out in the text, detachment may be caused by the halt transfer signal itself. Alternatively, polypeptides with sufficiently long segments synthesized after the halt transfer signal may cause steric hindrance and directly push the ribosomes off the membrane. The physical distance to which the ribosome can be removed from the membrane may of course be restricted by the attachment to the membrane of flanking ribosomes within a polysome. Only a small separation would, of course, be required to allow the looping of the polypeptide segment synthesized after the halt transfer signal. A third crossing of the membrane may occur if the halt transfer signal is followed by an interior cotranslational insertion signal (*g–i*). Multiple crossings may be generated by a sequence of alternating cotranslational insertion and halt transfer signals. It should be noted that the figure depicts only a single ribosome engaged in the act of translation. The different successive stages would take place simultaneously within a polysome. The location of the carboxy-terminal end of the polypeptide depends on whether the last transmembrane segment served as part of an insertion or a halt transfer signal. As a consequence of this model, cotranslational insertion signals span the membrane from the cytoplasmic to luminal side in the amino to carboxyl direction, whereas hydrophobic parts of halt transfer signals span the membrane with the opposite orientation.

on whether the last signal was a cotranslational insertion or a halt transfer signal. Theoretically, this type of mechanism could give rise to the existence of membrane-associated polypeptide molecules that by cotranslational or posttranslational proteolytic cleavage could produce combinations of three different

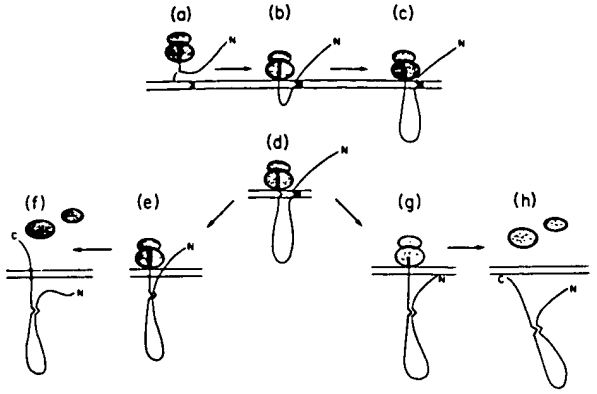


FIGURE 3 A possible mechanism for the translocation of an interior permanent cotranslational insertion signal and the preceding amino-terminal portions of a nascent polypeptide through the membrane. The affinity of cotranslational insertion signal (>) for the membrane (*a* and *b*) may be overcome by interaction with another portion of the polypeptide (>) (*d*) which facilitates passage of the entire molecule through the membrane (*g* and *h*) unless the vectorial discharge is interrupted by a subsequent halt transfer signal (*e* and *f*). Mechanisms of this sort may explain the vectorial discharge of proteins that do not have cleavable insertion signals, such as ovalbumin, or the transfer to the luminal side of the membrane of amino-terminal portions of membrane proteins, which occurs during the synthesis of rhodopsin and the p62 envelope glycoproteins of Sindbis and Semliki Forest virus (see Fig. 4).

types of proteins: secretory, membrane, and cytoplasmic.

Although the models described in Figs. 1 and 2 indicate that, in general, amino-terminal segments of mature proteins become exposed on the luminal side of the membrane only after cleavage of a preceding signal, this need not be an obligatory feature for all membrane or secretory proteins. This is demonstrated by the vectorial discharge of ovalbumin, which contains a functional signal (126) but is released into the microsomal lumen without proteolytic cleavage (157). If the insertion signal that ensures vectorial discharge of ovalbumin into the microsomal lumen is located in the interior of the polypeptide, as has been suggested (126), special mechanisms must ensure that sequences preceding the signal also pass through the membrane. A possible mechanism for such an effect is depicted in Fig. 3, where a stronger interaction between the cotranslational insertion signal and a subsequent region of the polypeptide serves to overcome the affinity of the signal for the membrane. This type of mechanism could account for the appearance of any uncleaved amino-terminal end of a polypeptide on the luminal side of the membrane, and therefore could apply to retinal opsin. This integral membrane protein is synthesized on bound polysomes but contains no transient cotranslational insertion signal (195); however, its amino-terminal segment bearing sugars (83, 65) is exposed on the intradiscal surface thought to represent the luminal side of the photoreceptor membrane.

Considerable insight into the biosynthetic process by which viral membrane glycoproteins are inserted into cellular membranes has been provided by studies with the closely related Sindbis and Semliki Forest (SFV) alpha viruses. The biogenesis of the envelope of these viruses provides examples in which several aspects of the theoretical model just described appear to operate. In these cases, all the structural viral proteins are derived from a single polyprotein encoded in a 26S mRNA molecule, which is translated using one initiation site (37, 204). For SFV, a viral capsid protein (C, 30,000 daltons) and two

viral membrane G (p62, 62,000 daltons, and E1, 49,000 daltons) are generated, in that order, by processing of the nascent polyprotein. The p62 glycoprotein is, in turn, a precursor of the envelope G proteins E2 (52,000 daltons) and E3 (10,000 daltons), which are derived from it by a later posttranslational cleavage (68, 205). Kinetic studies indicate that synthesis of the viral polyprotein begins in free ribosomes and that the capsid protein is proteolytically removed in the cytosol from the amino-terminal end of the nascent polypeptide chain (67) (Fig. 4*a*). The newly exposed amino terminus, corresponding to that of the p62 protein, appears to contain a signal for cotranslational insertion that then directs the binding of the ribosome to the ER membrane (Fig. 4*b*). Consequently, completion of the synthesis of p62, including core glycosylation, and synthesis and glycosylation of E1 takes place in bound polysomes. It should be noted (Fig. 4*j*) that after cotranslational insertion is completed, p62 and E1 appear also to remain attached to the membrane by sequences near their carboxy-terminal ends with the bulk of both proteins exposed on the luminal side of the ER membrane (69).

A similar situation is observed with Sindbis virus, in which association of the 26S messenger RNA with bound polysomes was demonstrated directly (246). In this case it was shown, using a temperature-sensitive mutant that fails to cleave off the capsid protein from the structural polyprotein, that this cleavage is necessary for insertion of the envelope glycoproteins into the ER membrane (247). More recently, direct sequence analysis of the amino-terminal region of the wild-type pE2 (the Sindbis equivalent of p62) synthesized in vitro in the presence of membranes and comparison of its primary structure with that of the product synthesized in vitro in the absence of membranes, from which the C protein is removed by a soluble enzyme, has suggested the existence of a permanent (noncleaved) amino-terminal cotranslational insertion signal in this polypeptide (20). The amino-terminal region of E2 must nevertheless itself be transferred across the membrane (Fig. 4*c*), since it is later released into the extracellular medium as a 9,800-dalton glycoprotein that is equivalent to the E3 virion G protein of SFV (240).

As discussed previously, the hydrophobic segments near the carboxy-terminal ends that anchor the viral glycoproteins to the membranes (69) most likely are part of the halt transfer signals that operate during translation. A halt transfer signal near the carboxy-terminal end of the p62 polypeptide of SFV would, according to the schemes presented in the figures, prevent the subsequent insertion of E1 sequences into the membrane. Two mechanisms for the insertion of the second G protein (E1) of SFV or Sindbis virus can then be envisaged. Wirth et al. (246) have proposed that insertion of E1 occurs after a second proteolytic cleavage of the polyprotein, which takes place on the cytoplasmic side of the membrane, exposes a new amino-terminal cotranslational insertion signal (Fig. 4*f*). These authors and Garoff et al. (67) have also considered the possibility that the second cleavage of the polyprotein may actually occur on the cisternal side of the ER, after the distal region of the nascent polyprotein has looped back into the membrane (Fig. 4*e*, *h*, and *i*). In this case, insertion of E1 would occur via what might be regarded as an internal cotranslational insertion signal in the polyprotein, which places the amino-terminal end of the E1 protein in the luminal side of the membrane. After two subsequent proteolytic cleavages, this part of the polyprotein would remain within the ER membrane in a transmembrane disposition. Recently, small membrane-associated polypeptides have been found in rough microsomal

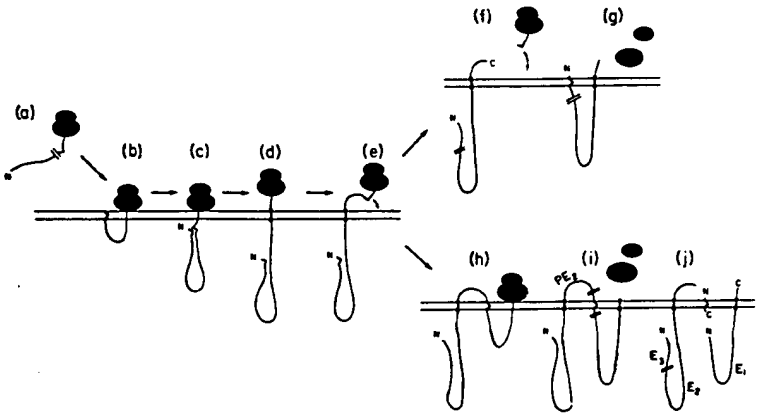


FIGURE 4 Schemes depicting the processing and subsequent insertion into the membrane of the nascent polyprotein precursor that yields the capsid and envelope glycoproteins of Sindbis and Semliki Forest viruses (adapted from the work of Garoff et al. [66]. The capsid protein is derived by proteolytic cleavage of the nascent polypeptide, the synthesis of which begins in the cytosol (*a*). This cleavage exposes a new amino-terminal cotranslational insertion signal (*b*), which is itself transferred across the membrane (*c*) and remains as a permanent feature of one of the viral proteins (E3). A subsequent halt transfer signal (*d*) determines that the carboxy-terminal end of pE2 or p62 (precursors of proteins E2 and E3) remains on the cytoplasmic side of the membrane. Reinsertion into the membrane may occur after cleavage of the precursor (*f*) generates a new amino-terminal cotranslational insertion signal, which, after a second cleavage (*g*), leaves the amino terminus of E1 on luminal side of the membrane. A halt transfer signal very near the carboxy-terminal end of the polyprotein determines the final orientation of E1. Alternatively, the insertion of E1 may occur without previous cleavage of the nascent chain through an internal cotranslational insertion signal that promotes reassociation of the ribosome with the membrane (*h*). Cleavage on both sides of the membrane generates a free carboxy-terminal end of pE2 on the cytoplasmic side and the amino-terminal end of E1 on the luminal side, with a small polypeptide spanning the membrane. A halt transfer signal near the carboxyl terminus of E1 ensures that this portion of the polypeptide remains on the cytoplasmic side of the membrane.

membranes from SFV- and Sindbis-infected cells (6,000 and 4,200 mol wt, respectively) (240, 241). These may represent the segments of the envelope polyproteins that in the primary translation products contain the interior insertion signals and are excised by proteolytic cleavages on both sides of the membrane.

Sorting-out Processes Must Follow Cotranslational Insertion into the ER

The diversity of ultimate destinations achieved by both luminal and membrane proteins released from bound ribosomes makes it necessary to invoke the existence of sorting-out processes that operate posttranslationally and recognize specific features (sorting-out signals) of each translation product that determine its subsequent pathway of circulation within the cell. Such sorting-out processes would control lateral displacement of proteins along membranes and their segregation in specific membrane domains as well as the flow of content within membrane vesicles and would therefore determine whether polypeptides are retained in the ER itself or are transferred to other membrane systems, including the Golgi apparatus, secretory granules, lysosomes, or specific domains of the plasma membrane.

A simple model for a sorting-out mechanism would involve structural features of all polypeptides discharged into the lumen of the ER which, in the absence of other specific signals, determine their complete passage through the secretory apparatus and discharge at the surface of the cell. Retention in a particular location along the pathway leading to plasma membrane and, when necessary, diversion from the secretory pathway would require ancillary signals. Thus, peripheral proteins of the luminal face of the ER membrane would contain retention signals that interact with luminally exposed segments of integral proteins or other already bound peripheral proteins, as has been shown for the mouse liver microsomal β-glucuronidase (224). Segregation of specific classes of proteins into membrane-bounded organelles derived from the ER, such as lysosomes, would require an initial interaction of these proteins with specific receptors exposed on the luminal faces of the ER or Golgi membranes. Subsequently, and possibly as a result of these interactions, such receptors bearing ligands would be collected in specialized areas to be incorporated into membrane vesicles that depart from the secretory pathway to reach their destination (58, 77). Mouse immunoglobulin light chain mutations have been described that appear to allow normal processing and discharge into the ER lumen but prevent secretion (142). Similarly, in humans a missense mutation (250) is known that markedly reduces the secretion of the serum protein α_1-antitrypsin (57) and produces its concomitant accumulation within the ER of liver cells (10). These mutations could affect specific signals that direct the protein along the secretory route or, according to the simple model just described, could simply alter certain general properties of the secretory polypeptides required for their transit through the cell (for example, solubility in the ER cisternal milieu).

It has long been recognized that, in contrast to cell sap proteins, secretory proteins, as well as many integral membrane proteins and lysosomal hydrolases, are glycosylated and contain variable amounts of carbohydrate residues bound to their polypeptide backbones. The possibility must therefore be considered that information for the sorting out of these three major classes of glycoproteins could be contained in their specific oligosaccharide configurations. Recent experiments have shown, however, that the presence of carbohydrate residues is not an obligatory requirement either for the secretion of proteins, as had been suggested by Eylar (56), or for the incorporation of integral membrane proteins into the plasma membrane. Thus, in cells treated with tunicamycin, a drug that inhibits the synthesis of oligosaccharide cores on lipid intermediates and therefore prevents the glycosylation of nascent polypeptides growing in bound polysomes, production of several secreted proteins proceeds at nearly normal rates (220). In some systems, however, such as immunoglobulin-producing cells, it has been reported (89) that tunicamycin markedly inhibits protein secretion. Since not all secretory proteins contain oligosaccharide chains (e.g., albumin) it is clear that the presence of a carbohydrate moiety is not required for secretion of a polypeptide. It is still possible, however, that, in some cases, the oligosaccharide linked to the polypeptide backbone may be necessary to maintain the protein in a conformation that is compatible with its transit through the secretory pathway.

Similarly, the presence of carbohydrates in viral envelope G proteins is not an absolute requirement for their transfer to the plasma membrane. Thus, substantial quantities of virions containing normal amounts of the VSV envelope G protein lacking carbohydrate may be produced in tissue culture cells treated with tunicamycin (72). These results indicate that carbohydrate moieties do not serve as signals directing the protein to the plasma membrane. On the other hand, tunicamycin treatment did lead to a pronounced inhibition of viral production, the extent of which varied with the viral strain and the temperature of incubation of the infected cells (71). This work demonstrated that the conformational properties of the G protein are affected by glycosylation (72) and that the decreased production of viruses in the presence of tunicamycin is likely to result from aggregation of the nonglycosylated protein at higher temperatures. Furthermore, VSV and influenza virions, which normally bud from only the basolateral and apical surfaces of polarized cultured epithelial cells (172), respectively, continue to display the polarized budding after glycosylation is inhibited by tunicamycin (78, 177), which indicates that even the finer degrees of segregation of membrane proteins can proceed in the absence of glycosylation.

A possible role for mannose-6-phosphate (Man-6-P) sugar residues in the sorting out of lysosomal hydrolases from secretory proteins within the ER or Golgi complex has been recently inferred from studies with cultured cells. It was first found by Neufeld and her associates (90, 191) that strains of genetically deficient fibroblasts (I cells) from patients with mucolipidosis II secrete lysosomal hydrolases into the medium rather than incorporating them into lysosomes. These enzymes, although enzymatically active, were found to lack a recognition marker for uptake by normal fibroblasts, whereas lysosomal hydrolases from normal cells could be taken up by a specific and saturatable process by both normal and I cell fibroblasts. These observations indicated that lysosomal hydrolases share a common recognition marker that is missing from the enzymes secreted by I cells and mediates their uptake by receptors found in the plasma membrane. Several lines of evidence indicated that phosphomannosyl residues represent the common feature recognized in lysosomal enzymes by the plasma membrane receptor. Uptake was found to be completely and specifically inhibited by Man-6-P (105) or by treatment of lysosomal enzymes with either alkaline phosphatase or endoglycosidase

H (58). Moreover, lysosomal enzymes have been shown to contain phosphorylated mannose residues that are missing from the hydrolases secreted by I cell fibroblasts (86). It was initially suggested that secretion and recapture of lysosomal hydrolases represented a major pathway for the incorporation of newly synthesized enzymes into lysosomes. This is unlikely, however, since addition of Man-6-P or antibodies against lysosomal enzymes to cells does not lead to accumulation of newly synthesized hydrolases into the culture medium (209, 235).

It has recently been found (87) that newly synthesized lysosomal enzymes exist intracellularly as larger precursor polypeptides that appear to be converted slowly by proteolytic cleavage to the mature enzymes. Hydrolases lacking the Man-6-P recognition marker are secreted by I cells in the precursor form. Secretion of precursor forms can also be induced by treatment of normal cells with the lysosomotropic drug chloroquine or with NH_4Cl, which alter the lysosomal pH. All these results suggest that the posttranslational modification of lysosomal hydrolase precursors by addition of phosphomannosyl residues to the oligosaccharide core, a process that is likely to occur in the Golgi apparatus (226), is necessary to divert the lysosomal enzymes from the secretory pathway and direct them to their destination (105). This is in accordance with a model in which, in the absence of retention signals, polypeptides discharged into the ER lumen are secreted from the cell, without the need of specific signals directing the protein along the secretory route. Release of hydrolase precursors from their receptors and maturation by proteolytic cleavage (77, 87) would occur under the pH conditions present within the lysosome.

The biogenetic scheme just discussed postulates the existence of intracellular receptors involved in the transport of lysosomal enzymes to their site of function. In fact, it has been determined that intracellular membranes contain four times as many receptor sites for lysosomal enzymes as does the plasma membrane and that the total number of receptors in a cell is insufficient to explain rates of pinocytotic uptake of lysosomal enzymes, unless the receptors are reutilized by means of a recycling process (58, 77). The secretion of lysosomal enzymes induced by chloroquine treatment and the impairment of uptake that is caused by this drug can both be explained by the failure of the receptors to release bound enzymes at the modified lysosomal pH, which leads to the unavailability of unoccupied receptors in the intracellular and surface membranes (77). An insufficient number of receptors may also account for the secretion of lysosomal hydrolase precursors bearing the Man-6-P marker from different cell lines under normal growth conditions.

Although a direct transfer of lysosomal enzymes from the Golgi apparatus to the lysosomes has not yet been directly demonstrated, it is currently thought that such a step is part of the major biogenetic route followed by the hydrolases (209). The presence of receptors on the plasma membrane could reflect their recycling through the surface before returning to the Golgi apparatus. It is still possible, however, as proposed by von Figura and Weber (235), who detected lysosomal enzymes at the cell surface by immunofluorescence, that hydrolases tightly bound to receptors must be brought to the plasma membrane before they are interiorized and incorporated into the lysosome. A pathway involving initial transfer to the plasma membrane would explain the partial secretion of hydrolases that is observed in normal cells, although in this model it must be assumed that addition of Man-6-P to the medium fails to displace the ligand from the receptor (77).

Although the evidence just discussed indicates that phosphomannosyl groups are necessary to effect the correct sorting out of lysosomal enzymes, it should be recognized that specific features of the polypeptide backbone must determine that these proteins are appropriate substrates for the enzymes responsible for this specific posttranslational modification. Furthermore, other features of the newly synthesized polypeptide, possibly even those contained within peptide segments that are removed from precursors by proteolysis, may also be necessary for transfer of the hydrolases to the lysosomes.

Many secretory polypeptides such as albumin, insulin, collagen, and parathyroid hormones (cf. 149) also exist intracellularly in precursor forms (prosecretory proteins) containing peptide segments later removed by proteolysis. This raises the possibility that information within the excised segments may serve to direct the proteins through the secretory pathway. However, the presence of propieces in intracellular precursors is not a general feature of all secretory polypeptides. Furthermore, the removal of propieces appears not to be necessary for secretion, since an abnormal mutant form of human proalbumin lacking the protease recognition site is secreted normally into the bloodstream (26).

The preceding discussion on possible sorting-out mechanisms for proteins discharged into the ER lumen led us to invoke interactions of specific signals with membrane receptors which themselves must be segregated in different domains of intracellular or surface membrane systems. An economic mechanism for the segregation of specific membrane proteins would be one involving the recognition of common features within classes of polypeptides with the same destination. In this regard it is important to note that in simple transmembrane proteins, such as the G proteins of enveloped viruses, three distinct polypeptide segments can be recognized that can serve as sorting-out signals. One of these contains hydrophobic amino acids and lies within the phospholipid bilayer, whereas the others are exposed on the cytoplasmic and luminal faces of the membrane. Accumulation of integral membrane proteins in specific domains of a membrane system may result from lateral interactions involving any of these three types of segments, or from associations mediated by peripheral membrane proteins on either side of the membrane. In particular, segments exposed on the cytoplasmic side of the membrane may establish directly or indirectly an interaction with cytoskeletal elements responsible for the transfer of the polypeptide between subcellular compartments. If this is the case, then the transport of polypeptides such as E1 of SFV, which has only three residues exposed on the cytoplasmic side of the membrane (66), would require their oligomeric assembly with other transmembrane proteins.

Transfer of membrane and content proteins between subcellular compartments is thought to proceed through the formation of vesicles that bud from one type of membrane and fuse with another (155). Evidence has accumulated indicating that vesicles coated with a regular basket of the 180,000-dalton polypeptide clathrin (159) are intermediates in the transfer of macromolecules between subcellular compartments such as the ER and Golgi apparatus (103), and are involved in the transport of proteins to the plasma membrane (63) and in the uptake of macromolecules by receptor-mediated endocytosis (e.g., 74, 178). A recent study of the pathway followed in virus-infected cells by the newly synthesized VSV G protein indicates that clathrin-coated vesicles are involved in the transfer of this

polypeptide from the ER to the Golgi apparatus and from there to the plasma membrane (179, 180). Coated vesicles were prepared from tissue culture cells at different times after labeling with [^{35}S]methionine in a pulse-chase experiment. Labeled G_1, the high mannose form of the G protein, was found in vesicles isolated a short time after labeling, whereas labeled, terminally glycosylated, mature G_2, which is insensitive to endoglycosidase H, appeared in the coated vesicles only at later times during the chase. Since the G_1 form of the G protein is produced in the ER as a result of cotranslational cleavage of the insertion signal and core glycosylation, whereas conversion of G_1 to G_2 is expected to occur in the Golgi apparatus, these results were interpreted to reflect the participation of coated vesicles at two stages in the transit of the G protein from its site of synthesis to the plasma membrane. The G_1-containing vesicles presumably represent the elements transferring the glycoprotein from the ER to the Golgi apparatus, whereas the G_2-containing coated vesicles would represent the carriers that bring the mature glycoprotein from the Golgi apparatus to the cell surface. Preliminary evidence was presented that the two classes of coated vesicles are structurally distinct since they could be partially separated by density gradient centrifugation and could be distinguished by their susceptibility to immune precipitation with anticlathrin antibodies (179).

It would be of great interest to understand the mechanism that determines the formation of clathrin-coated vesicles from specific regions of the ER and Golgi membranes. Although no direct data on this question are available, by analogy with the formation of coated vesicles during receptor-mediated endocytosis (29), it can be proposed that cytoplasmic segments of clusters of transmembrane proteins provide sites for clathrin recognition and the initiation of basket assembly and vesicle formation. The question remains, however, for receptor-mediated endocytosis as well as for intracellular transport via coated vesicles, of which features of different membrane proteins may be recognized by clathrin to initiate formation of coated vesicles. Furthermore, nothing is known of how delivery to a specific accepting membrane is controlled. Sorting-out signals of the membrane proteins contained within the vesicles could, of course, play a determinant role in this process.

Although few data are currently available on the nature of sorting-out processes, speculative considerations such as those just presented are necessary to provide a conceptual framework for further investigations in this area, which is central to the problem of organelle biogenesis. There is, of course, a great need to accumulate information on primary sequences of organellar and membrane polypeptides. Such data are rapidly emerging as the result of advances in protein chemistry and in recombinant DNA and nucleotide sequencing techniques. When the structures of the corresponding domains of transmembrane proteins are compared with each other, one may be able to detect features that are likely candidates for the sorting-out signals that determine segregation in different compartments. Definitive identification of sorting-out signal segments within membrane and organelle polypeptides will ultimately require the production of proteins bearing site-specific mutations and the synthesis of chimeric proteins containing selected arrays of different putative signal segments, experimental approaches that have already been applied to studies of insertion signals (53, 140, 227, 228). Application of recombinant DNA techniques to eucaryotic cells using newly developed vectors and transformation techniques that lead to substantial levels of expression of exogenous artificial genes, together with refined methods of cell fractionation and immunocytochemistry, is likely to play a major role in furthering our understanding of the sorting-out processes involved in organelle biogenesis.

Posttranslational Transfer of Polypeptides to Their Sites of Function

It is important to emphasize that synthesis in bound polysomes with its concomitant cotranslational insertion into a membrane is not the only mechanism by which polypeptides can be transferred across or incorporated into the membranes of eucaryotic cells. It is now evident that many proteins synthesized on free polysomes and discharged into the cell sap are subsequently taken up into specific organelles.

The majority of proteins within mitochondria and chloroplasts, for example, are coded for by nuclear genes and are synthesized on cytoplasmic polyribosomes (35, 193). For several such proteins it has been shown that incorporation into the organelle occurs posttranslationally and, therefore, free polypeptides must in some way pass through the limiting membranes (34, 82, 84, 91, 128, 129, 165). Such polypeptides must contain structural features, which may be designated as "primary organellar addressing signals," that serve to determine or to stabilize a posttranslational interaction with specific components of the receptor membrane. It may be suggested that polypeptides within families with the same organellar destination contain a common primary addressing signal for the initial interaction with a single receptor in the cytoplasmic surface of the membrane limiting that organelle. Once the posttranslational association of a polypeptide with the organellar membrane has been established, mechanisms that involve an energy-consuming system (translocator), conformational changes and/or proteolytic processing may assist in the subsequent passage of the polypeptide through the membrane.

We have recently identified a peptide segment within apocytochrome *c* that appears to contain the primary addressing signal that determines the uptake of this protein into mitochondria, after the newly synthesized polypeptide is released from free polysomes (133). Cytochrome *c* was synthesized in vitro in translation mixtures programmed with rat liver mRNA from animals treated with triiodothyronine, in which levels of the specific mRNA are increased. It was found that the in-vitro-synthesized product does not differ in primary sequence from the mature polypeptide, except that it retains the initiator methionine. This suggested that components of the signal for mitochondrial uptake of apocytochrome *c* are contained in the mature polypeptide. When liver mitochondria (but not mitoplasts devoid of the outer organellar membrane) were added to the cell-free system after translation was completed, the newly synthesized cytochrome *c* became inaccessible to digestion by exogenous proteases and was recovered with the sedimentable mitochondrial fraction. A similar posttranslational uptake has been reported for *Neurospora* cytochrome *c* (110, 251). The specificity of the posttranslational uptake of rat liver cytochrome *c* was demonstrated by the fact that added apocytochrome *c*, but not the native holocytochrome, could compete with the in-vitro-synthesized product for its incorporation into mitochondria. Competition experiments using separated cyanogen bromide fragments of apocytochrome *c* revealed that the signal for posttranslational uptake resides within a segment of the polypeptide that extends from residue 66 to the carboxy-terminal end, since only this segment could compete with and, at appropriate concentrations, completely prevent the entrance of the in vitro product into the organelle. It is interesting to

note that the segment containing the putative signal for mitochondrial uptake includes that portion of cytochrome *c* that is most highly conserved in evolution (132, 232). The failure of the native holocytochrome to compete with the in vitro product for a hypothetical receptor on the mitochondrial surface suggests that acquisition of the heme leads to sequestration of the signal segment within the protein molecule and that the final folding of the polypeptide mediated by heme binding occurs within the mitochondria.

With the exception of cytochrome *c* and the ADP-ATP translocators of the inner mitochondrial membrane (133, 251, 252), for which high molecular weight precursors have not been detected, studies with other mitochondrial polypeptides of cytoplasmic origin, such as subunit V of cytochrome bC_1 (39), cytochrome *c* peroxidase (129), citrate synthase (85), subunits of cytochrome oxidase (171), and ATPase (120, 128, 192), as well as polypeptides of the mitochondrial matrix such as carbamylpnosphate synthetase (141, 203) and ornithine transcarbamylase (38), have shown that these polypeptides are released from cytoplasmic polysomes, as precursors that are proteolytically processed upon entrance into the organelle. A similar mechanism appears to operate in chloroplasts. For example, it has been demonstrated that the small subunit of ribulose-1,5-bisphosphate carboxylase, which is a chloroplast polypeptide synthesized on free cytoplasmic polyribosomes (48, 91), undergoes posttranslational proteolytic removal of an amino-terminal segment, 44 amino acids in length, during or immediately after its incorporation into the organelle (34, 48, 91, 196). The endopeptidase that effects the proteolytic cleavage is apparently a soluble enzyme located within the chloroplast (34, 91). The suggestion was made (196) that the transient segment, which does not contain the cluster of hydrophobic amino acid residues characteristic of signals for cotranslational insertion, represents a "transit sequence" that mediates the recognition of the newly synthesized polypeptide by membrane receptors and its subsequent transport into the chloroplast stroma. It should be noted, however, that segments removed by proteolytic cleavage during the posttranslational uptake of polypeptides synthesized in free polysomes may not themselves necessarily represent the primary organellar addressing signals that interact directly with organelle receptors. If this were the case, transient amino-terminal segments, like the one in precursor of the small subunit of ribulose-1,5-bisphosphate carboxylase, would be recognized in nascent polypeptides and would lead to association of ribosomes synthesizing these polypeptides with the outer organellar membrane. Instead, it may be proposed that peptide segments removed after entrance into the organelle serve to maintain the polypeptide released from the ribosome in a configuration in which a true signal, to which other features of the polypeptide may contribute, is recognizable by the organelle surface receptors. The change in conformation that results from removal of the terminal peptide and leads to the functional form of the protein may, of course, also play a role in the retention of the polypeptide within the organelle.

The presence of a transient peptide may not always be required to maintain a newly synthesized polypeptide in a conformation capable of interacting with the organelle receptors. In the case of cytochrome *c*, for example, the necessary conformation is present in the apocytochrome before acquisition of the heme, and a conformational change occurs upon entrance into the organelle without the need for proteolysis. The notion that, when present, the transient segment serves to maintain a newly synthesized polypeptide in a conformation that is recognized by the specific membrane receptor resembles aspects of the "membrane trigger folding hypothesis" of Wickner (243), who emphasized the possible role of transient peptide segments in altering the folding pathway of a newly synthesized polypeptide.

It has been shown that posttranslational maturation of polypeptides destined to the chloroplast stroma and the inner chamber of mitochondria requires the expenditure of energy (80, 148). It remains to be determined whether energy is required for the operation of the translocation mechanisms that effect passage of the polypeptides through the membranes or for subsequent modifications that lead to the functional configuration of the proteins, and possibly render the uptake process irreversible.

It has recently been shown that transmembrane insertion and posttranslational maturation of a precursor of the M13 phage coat protein destined for the cytoplasmic membrane of *E. coli* require the maintenance of an electrochemical potential across the membrane (42, 43). This was inferred from the observations that these processes are prevented by metabolic poisons that uncouple oxidative phosphorylation but not by arsenate, which depletes the cellular pool of high-energy phosphate bonds but does not dissipate the electrochemical gradient (43).

Studies on the biosynthesis of the peroxisomal enzymes urate oxidase and catalase have shown that these proteins are synthesized in free polysomes (73, 112). Thus, the completed polypeptides must also cross an organellar membrane posttranslationally but it is yet to be demonstrated whether they are incorporated directly into preexisting peroxisomes or into developing forms that may bud from the ER. The occurrence of posttranslational modifications has not been documented in these cases.

Further studies to identify and characterize the signals that determine posttranslational uptake of polypeptides and the receptors and proteases that must be associated with the limiting membranes of mitochondria, chloroplasts, and possibly peroxisomes, are necessary to understand the processes that allow translocation of families of completed polypeptides from the cell sap to these specific subcellular compartments.

The large number of different polypeptides synthesized by free cytoplasmic ribosomes that must reach the various subcompartments of mitochondria and chloroplasts raises the possibility that common sites of entry are used for the posttranslational uptake of the resident polypeptides of one or more subcompartments. By analogy with events following cotranslational insertion into the ER, the initial uptake of a polypeptide into the organelle by a common mechanism could then be followed by its sorting out to one of the various subcompartments within the organelle.

The existence of posttranslational translocations of polypeptides into semiautonomous organelles raises the question of whether a similar mechanism could be utilized for the insertion of at least some proteins into the ER, Golgi complex, and plasma membrane systems, organelles for which cotranslational insertion has been demonstrated or is thought to play the major role. Although the proposal has been made (183) that secretory proteins may be discharged into the cell sap, in which case secretion would require only a direct crossing of the plasma membrane, no well-documented case of posttranslational transfer across or transmembrane insertion of a polypeptide into the ER, Golgi complex, or plasma membrane has

yet been provided.

Current notions on the mechanism of protein glycosylation appear to exclude the possibility that glycoproteins containing asparagine-linked carbohydrates are posttranslationally inserted into membranes since nascent polypeptides seem to be the substrate required by membrane-associated enzymes for the transfer of a core oligosaccharide from a lipid intermediate to an exposed AsN-X-(Ser-Thr) sequence in the polypeptide chain (219). For nonglycosylated integral membrane proteins, however, a posttranslational translocation or transmembrane insertion into ER, Golgi complex, and plasma membrane systems would remain theoretically possible, if appropriate receptors or translocators existed in these membranes to ensure the specific subcellular distribution and asymmetric orientation. In fact, our work with the Na^+,K^+-ATPase of the plasma membrane indicates that the large nonglycosylated subunit of this protein is synthesized in free polysomes and is discharged in the cell sap before its ultimate incorporation into the plasma membrane. The glycosylated small subunit of the ATPase is, however, synthesized in the rough endoplasmic reticulum and interaction between the two subunits may be an important event in the posttranslational assembly of the mature protein within the membrane (201).

Several ER integral membrane proteins such as cytochrome b_5 and NADH cytochrome b_5 reductase that are exposed on the cytoplasmic membrane face and do not appear to have a transmembrane disposition have recently been demonstrated to be synthesized in free polysomes (23, 75, 151, 167) and must therefore be inserted posttranslationally into their membrane sites of function. These proteins contain hydrophobic segments located near the carboxy-terminal end (153) that are likely to serve as posttranslational insertion signals responsible for the permanent association of the polypeptides with the ER membrane. Although no experimental evidence has been provided, it can be presumed that the association of the polyoma middle T antigen with the plasma membrane also occurs posttranslationally. The nucleotide sequence of the gene indicates the presence of a single hydrophobic segment in the polypeptide located near the carboxy-terminal end (215).

As mentioned previously, substantial evidence has recently been presented that in vivo the procoat protein of the coli phage M13 of *E. coli* is synthesized in free polysomes and is inserted into the cytoplasmic membrane posttranslationally (98, 99). Although Wickner and his colleagues (244) have also provided evidence that such posttranslational insertion can be effected in vitro after synthesis is completed, the experiments of Chang et al. (31) led to the opposite conclusion. They indicated that in vitro insertion of the M13 coat protein in the proper transmembrane orientation, with concomitant cleavage of the leader segment, occurred only when inverted cytoplasmic membrane vesicles were present during polypeptide synthesis and not when they were added after synthesis was completed.

In a recent review, Wicker (243) summarized a number of cases in which soluble secreted proteins can later become incorporated into or traverse cellular membranes in order to carry out their functions. Such examples include the membrane attack complex of complement (163), the mellitin component of bee venom (245), and bacterial toxins (239). He also mentions instances in which proteins may have been assembled asymmetrically into phospholipid bilayers of vesicles formed during membrane reconstitution experiments. On the basis of these observations and his studies on the posttranslational incorporation of the M13 procoat protein into the *E. coli* cytoplasmic membrane, Wickner has proposed a generalized model for the incorporation of proteins into membranes that does not require coupling to protein synthesis or the presence of a proteinaceous pore in the membrane. In this "membrane trigger folding hypothesis" (243), proteins encounter their target membranes during or shortly after their synthesis, after they have become folded in a manner compatible with the aqueous environment to which they are first exposed. This encounter would trigger the refolding of the protein into a conformation that exposes hydrophobic regions to the hydrophobic core of the membrane and allows penetration of the polypeptide through the phospholipid bilayer.

According to Wickner's model, transient peptide segments in precursor forms would serve mainly to alter the folding pathway of the polypeptide, which is modified when the polypeptide enters the phospholipid bilayer to achieve the final conformation. Although in this model a specific receptor protein may or may not be involved in facilitating the insertion process, the final conformation of the protein would be determined solely by the primary amino acid sequence and, one would presume, by the composition of the lipid bilayer and, possibly, interactions with other proteins.

In the membrane trigger hypothesis, removal of the transient segment is envisaged to permit the proteins to function or "to drive the assembly reaction." In this regard, a recent example has been presented of a conformational difference between precursors and mature forms of a single membrane protein. The unprocessed precursor of the leucine binding protein of the *E. coli* membrane was found to be much more sensitive to proteolytic cleavage at sites located within the body of the molecule than the mature protein (152).

Wickner has argued (243) that cotranslational insertion cannot be an important means of ensuring the transverse disposition of proteins that cross the membrane more than once or have their amino-terminal ends exposed on the cytoplasmic face of the membranes and in his view only the thermodynamics of protein folding governs the assembly of a protein in a membrane. The more generalized signal hypothesis presented in this paper, however, clearly accounts for various transverse dispositions of polypeptides as well as for more than one crossing of the membrane. Such dispositions are the direct result of the mechanism of cotranslational insertion, if amino-terminal and interior insertion signals, as well as halt transfer signals, are assumed to be present more than once in a single polypeptide.

It should be noted that the class of proteins that is inserted cotranslationally into ER membranes, including secretory, membrane, and lysosomal polypeptides, clearly shows a behavior different from that expected from Wickner's model. The transient amino-terminal peptides of these proteins may alter the folding pathway of the polypeptides, but, in contrast to Wickner's prediction, if synthesis is completed in an aqueous environment, the folded preproteins cannot enter the membrane. The behavior of these polypeptides is better explained by assuming that the leader segments serve as signals to establish an association of the ribosome with the membrane that ensures that complete folding does not take place in the aqueous environment of the cytoplasmic side. Thus, at least for certain portions of the polypeptide, folding could take place, from the very beginning, in the hydrophobic environment of the membrane, or after the polypeptide reaches the luminal side.

In considering mechanisms for the posttranslational insertion of some transmembrane proteins into membranes, Singer (207) has paid special attention to the transmembrane insertion of

oligomeric proteins that form channels for ion transport across membranes. He suggested that, posttranslationally, single subunits of these proteins could first become partially embedded in the cytoplasmic side of the membrane and that, subsequently, the spontaneous dimerization of these polypeptides would form a channel initially located entirely on that side of the membrane. To explain passage across the membrane, Singer invoked hydrophobic processing of amino acid residues on the exposed faces of this dimer by reactions that would eliminate polar and ionic groups and facilitate penetration of the aggregate through the phospholipid bilayer. It was further assumed that hydrophilic processing by glycosylation of residues emerging on the other side of the membrane could serve to pull more of the polypeptide through the membrane. The latter aspect of this model recalls Bretscher's (28) earlier proposal of a role of glycosylation in stabilizing the transmembrane disposition of membrane proteins, which were assumed to be synthesized in free polysomes and to become originally inserted into membranes spontaneously. As indicated previously, multimeric transmembrane proteins containing nonglycosylated subunits, such as the Na^+,K^+-ATPase of plasma membranes (201), offer the opportunity to study mechanisms by which polypeptides with a transmembrane disposition may be incorporated posttranslationally into functional membrane-bound complexes.

A Scheme for an Evolutionary Relationship between the Processes of Membrane Biogenesis and Secretion

The previous discussion has emphasized extensive similarities between the biogenetic pathways for secretory and membrane proteins. It appears unlikely that so similar strategies as are used by these two classes of proteins for traversing or penetrating cellular membranes and for circulation within the cell (60, 155, 181, 188) have developed independently during the course of evolution. In an early discussion of the role of membrane-bound ribosomes in the synthesis of secretory and ER membrane proteins, Palade (154) intimated that some ER membrane proteins may have served as "ancestral models" for secretory proteins. Indeed, it is reasonable to assume that membrane and secretory proteins, as we know them today, represent elaborations on different stages of a progressive evolutionary development that first led to the insertion of cytoplasmic proteins into membranes and later to the extrusion of portions of these proteins from the cell and their release from the membrane by proteolytic cleavage.

In this regard it should be noted that membrane proteins are likely to have been present in even the most primitive cells, where they participated in essential functions such as nutrient and ion transport, electron transport, and enzymatic catalysis of reactions that require hydrophobic environments. On the other hand, secretion, although a widespread and important biological phenomenon, is likely to have first represented only a specialized function of some cells, appearing as a relatively late development in the course of evolution. These considerations lead us to propose what may be regarded as a plausible evolutionary scenario for the origin of secretory proteins and for the development of a secretory apparatus.

In this scheme, we first consider a primordial cell type (Fig. 5*I*) that has no intracellular membrane-bounded organelles and is limited by a simple phospholipid bilayer. It may be assumed that the presence of hydrophobic regions in some of the proteins released by ribosomes, or their primitive counterparts, into the intracellular milieu led to their subsequent incorporation into the cytoplasmic face of the hydrophobic boundary layer and thus to the development of a primitive membrane (Fig. 5*I*). No specific mechanism for such an insertion developed, however, until proteins were synthesized

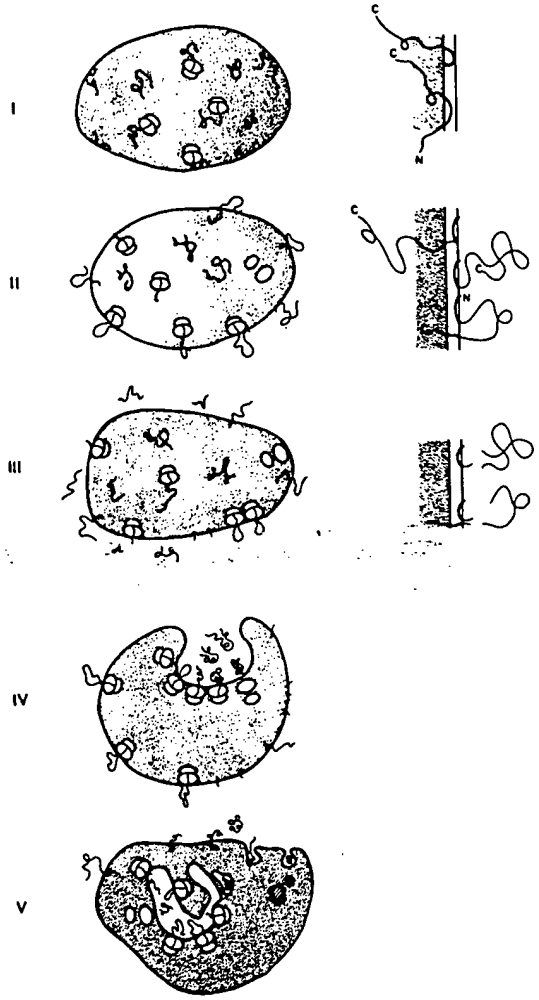


FIGURE 5 An evolutionary scenario for the origin of secretory polypeptides from ancestral membrane proteins and the subsequent development of a secretory apparatus. It is presumed in a primordial cell (*I*) the association of proteins with the lipid membrane was a posttranslational event determined by hydrophobic segments within the proteins. Hydrophobic segments, located sufficiently near the amino-terminus of the protein, led to association of nascent polypeptides with the membrane, an event that facilitated traversal of the hydrophobic barrier during polypeptide synthesis before the protein achieved its final folding (*II*). This process generates the first transmembrane proteins. Proteolysis and shedding of exposed segments of transmembrane proteins marks the evolutionary origin of secretion (*III*). The drawings to the right of *I*, *II*, and *III* represent magnifications showing the spatial disposition of membrane proteins in the bilayer. The shaded area corresponds to the cytoplasm. The appearance of ribosome binding sites in the membranes ensures the coupling of protein synthesis and vectorial discharge. The capacity for secretion is enhanced by the development of membrane invaginations (*IV*), which makes posttranslational modifications possible and allows for the storage of secretory products. Ribosome binding sites then become restricted to invaginated areas of the plasma membrane which, in the final stages of evolution, give rise to the endoplasmic reticulum (*V*). Membrane channels that serve to connect this proto ER with the cell surface represent possible precursors of the Golgi apparatus.

that contained hydrophobic regions located sufficiently near the amino-terminal end of the polypeptide so as to become exposed to the intracellular environment before translation was completed (Fig. 5 *II*). This would have resulted in the association of the ribosomes themselves with the membrane via their nascent chains, and therefore in the coupling of the processes of translation and insertion of the polypeptide into the phospholipid bilayer. Because some polypeptides were inserted into the membrane during their synthesis, before they were completely folded in an aqueous environment, the association of the ribosomes with the membrane may have facilitated the traversing of the phospholipid barrier and the appearance of the first transmembrane proteins, which marked the emergence of a true plasma membrane. A most important consequence of the generation of transmembrane proteins is that only cleavage of portions exposed on the cell surface, effected by environmental conditions or by putative membrane-associated proteases, was required for the shedding of polypeptide segments that represented the origin of secretion (Fig. 5 *III*).

The ability to shed polypeptide fragments is likely to have conferred great evolutionary advantages to secreting cells, which in this way became able to modify their environment. Primordial secreted polypeptides may have exerted physical effects and allowed for a primitive regulation of the osmotic pressure and surface tension. More importantly, they are likely to have served biological functions such as acting as scavenger enzymes that derived nutrients from surrounding macromolecules, as proposed for the most primitive heterotrophes by deDuve and Wattiaux (45). It is reasonable to assume that to enable a more effective secretion by shedding, the hydrophobic segment, which originally served as a primitive signal for insertion of the nascent protein into the membrane, was in later stages in evolution transferred to the amino-terminal end of the polypeptide by genetic mechanisms involving DNA translocation. The effective result of this terminalization of the primitive signal would have been the formation of extremely amphipathic molecules (equivalent to present presecretory proteins) that served as ideal substrates for cleavage into two independent parts: hydrophobic ones, which remained membrane-associated, and hydrophilic portions, which were released into the environment.

The selective advantages conferred by secretion must have also resulted in the elaboration of a more efficient secretory apparatus, which, according to the previous scheme, must have been derived from the plasma membrane. One early development may have been the appearance of ribosome receptors on the membrane that allowed for a direct tight binding to the membrane of the ribosomes engaged in the insertion of their nascent polypeptides. This should have facilitated the insertion process by coupling the energy of protein synthesis to the insertion, and contributing to this process the energy of binding of the ribosomes to the receptors.

Another elaboration that would have enhanced the cellular capacity for secretion is the formation of plasma membrane invaginations that enlarged the membrane surface available for ribosome binding. A secondary, but important consequence of the appearance of a membrane invagination (Fig. 5 *IV*) is the creation of a pouch in which shed portions of surface molecules could be collected before discharge into the environment. deDuve and Wattiaux (45) have envisioned that the development of a pouch in the early stages of evolution of eucaryotic cells allowed captured food and secreted enzymes to be trapped together and therefore proposed that it was followed by the formation of an endocytic vacuole, which represented the earliest form of lysosomes. The creation of a pouch that ultimately became sealed off from the surface, forming a primitive secretory vacuole, may have had additional profound evolutionary consequences by radically changing the character of the secretory process. Secretory discharge needed no longer be a continuous event that fatally followed cleavage of the polypeptide. Instead, mechanisms for its regulation could evolve that converted it into a flexible response controlled by stimuli, which either led to the opening of the communication between the pouch and the cell surface or regulated the fusion of the membrane of the primitive secretory vacuole with the plasma membrane. Furthermore, the dissociation between synthesis of polypeptides and their release to their environment created the possibility of posttranslational modifications of the secretory products by enzyme systems located in the invaginated membranes. Because of the evolutionary advantages of these posttranslational modifications, the partial internalization of ribosome binding sites that resulted from the early invagination is likely to have been followed by a strict segregation of binding sites to the invaginated membranes, which, when independent from the cell surface, became a proto-ER as well as a primitive secretory vacuole. A concentration of ribosome binding sites in the deeper portions of the endoplasmic membrane system is likely to have ensued since it introduced a definite vectorial character to the pathway of circulation of secretory polypeptides within the cell. It could be hypothesized that because newly synthesized polypeptides entering into the ER must traverse the rest of the system to reach the cell surface, ordered sequences of posttranslational modifications became possible and compartmentalization of the corresponding enzymes in different regions of the endoplasmic membrane system occurred. The smooth ER and Golgi apparatus may indeed be considered evolutionary elaborations on membrane channels that served to link deeper portions of the invaginations bearing ribosomes with the cell surface. These developments may have enabled the segregation of certain classes of secretory proteins into primitive primary lysosomes, whereas others could be concentrated in granules to be secreted at the cell surface by exocytosis.

In this evolutionary view, secretion is the function that sustained the development of an intracellular membrane system, and the rough ER plays a critical role in the development of several other organelles that are part of the endoplasmic membrane system of the cell. In the final stages of the proposed evolutionary process, the different compartments of the endoplasmic system that are derived from the plasma membrane do not communicate permanently with each other, and specific recognition systems between the different membranes developed that enabled the transfer of material from one compartment to another, storage in secretory granules or in primary lysosomes, and discharge to selected areas of plasma membrane (either interiorized in the form of phagocytic vacuoles or at the cell surface), while the rest of the system remained sealed off from the environment.

Although the evolutionary process just described provides a possible explanation for the origin of the secretory apparatus, it also implies that membrane proteins had the opportunity to become substrates for posttranslational modifications that may have evolved for or become characteristic of secretory proteins, and vice versa. This might have accelerated the development of new functional properties for both types of proteins, with selective advantages for the cell.

Finally, it should be noted that although a narrow interpretation of the evolutionary scheme proposed would suggest that

all secretory proteins are derived from ancestral membrane proteins, with the cotranslational insertion signal representing a trace of their evolutionary history, one cannot ignore the possibility of frequent genetic recombination events generating new secretory or membrane proteins by linking signal segments to preexisting cytoplasmic proteins, in a manner similar to that effected in the laboratory through genetic engineering manipulations (199, 227, 228). In addition, the existence of mechanisms for splicing together noncontiguous segments of RNA molecules during the processing of messenger RNA precursors creates the possibility of linking a single cotranslational insertion, halt transfer, sorting-out, or primary organellar addressing signal to several proteins with different functions or, conversely, for generating single functional activities with multiple cellular locations.

The suggestion that secretory proteins originated as shed fragments of membrane proteins raises the possibility that even in the contemporary biosphere some secretory polypeptides are derived by proteolysis from existing membrane proteins. The proposed evolutionary relationship between membrane proteins and secretory proteins also suggests the possibility that complementary structural features of specific secretory and membrane polypeptides that were once contiguous in a primitive (ancestral) transmembrane protein are still manifested in functional interactions between their evolutionary derivatives. We may suspect, for example, that such a relationship exists for some peripheral and integral membrane proteins that form complexes at the cell surface, such as β_2-microglobulin and the heavy chain component of the histocompatibility antigen, or between some polypeptide hormones and their membrane receptors.

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REFERENCES

1. Adams, A. J., M. Tanaka, and H. Shichi. 1978. Concanavalin A binding to rod outer segment membranes: usefulness for preparation of intact disks. *Exp. Eye Res.* 27:595–605.
2. Adelman, M. R., G. Blobel, and D. D. Sabatini. 1973. An improved cell fractionation procedure for the preparation of rat liver membrane-bound ribosomes. *J. Cell Biol.* 56: 191–205.
3. Adelman, M. R., D. D. Sabatini, and G. Blobel. 1973. Ribosome-membrane interaction. Nondestructive disassembly of rat liver rough microsomes into ribosomal and membranous components. *J. Cell Biol.* 56:206–229.
4. Bainton, D. F., and M. G. Farquhar. 1970. Segregation and packaging of granule enzymes in eosinophilic leukocyte. *J. Cell Biol.* 45:54–73.
5. Bar-Nun, S., G. Kreibich, M. Adesnik, L. Alterman, M. Negishi, and D. D. Sabatini. 1980. Synthesis and insertion of cytochrome P-450 into endoplasmic reticulum membranes. *Proc. Natl. Acad. Sci. U. S. A.* 77(#2):965–969.
6. Bassford, P., and J. Beckwith. 1979. *Escherichia coli* mutants accumulating the precursor of a secreted protein in the cytoplasm. *Nature (Lond.).* 277:538–541.
7. Bassford, P. J., T. J. Silhavy, and J. Beckwith. 1979. Use of gene fusion to study secretion of maltose-binding protein into *Escherichia coli* periplasm. *J. Bacteriol.* 139:19–31.
8. Beckwith, J., T. Silhavy, H. Inouye, H. Shuman, M. Schwartz, S. Emr, P. Bassford, and E. Brickman. 1978. The mechanisms of localization of proteins in *Escherichia coli*. *In* transport of Macromolecules in Cellular Systems. S. C. Silverstein, editor. Dahlem Konferenzen, Berlin. 299–314.
9. Bedouelle, H., P. J. Bassford, Jr., A. V. Fowler, I. Zabin, J. Beckwith, and M. Hofnung. 1980. Mutations which alter the function of the signal sequence of the maltose binding protein of *Escherichia coli*. *Nature (Lond.)* 285:78–81.
10. Berg, N. O., and S. Eriksson. 1972. Liver disease in adults with alpha$_1$-antitrypsin deficiency. *New Engl. J. Med.* 287:1264–1267.
11. Bergeron, J. J. M., M. V. Berridge, and W. H. Evans. 1975. Biogenesis of plasmalemmal glycoproteins. Intracellular site of synthesis of mouse liver plasmalemmal 5′-nucleotidase as determined by the subcellular location of messenger RNA coding for 5′-nucleotidase. *Biochim. Biophys. Acta* 407:325–337.
12. Bergman, L. W., and W. M. Kuehl. 1979. Co-translational modifications of nascent immunoglobulin heavy and light chains. *J. Supramol. Struct.* 11:9–24.
13. Biellinska, M., G. Rogers, T. Rucinsky, and I. Boime. 1979. Processing *in vitro* of placental peptide hormones by smooth microsomes. *Proc. Natl. Acad. Sci. U. S. A.* 76: 6152–6156.
14. Blobel, G. 1978. Mechanisms for the intracellular compartment of newly synthesized proteins. *FEBS (Fed. Eur. Biochem. Soc.) Proc. Meet.* 43(A2):99–108.
15. Blobel, G. 1980. Intracellular protein topogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 77(3): 1496–1500.
16. Blobel, G., and B. Dobberstein. 1975. Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* 67:835–851.
17. Blobel, G., and B. Dobberstein. 1975. Transfer of proteins across membranes. II. Reconstitution of functional rough microsomes from heterologous components. *J. Cell Biol.* 67:852–862.
18. Blobel, G., and D. D. Sabatini. 1970. Controlled proteolysis of nascent polypeptides in rat liver cell fractions. I. Location of the polypeptides within ribosomes. *J. Cell Biol.* 45: 130–145.
19. Blobel, G., and D. D. Sabatini. 1971. Ribosome-membrane interaction in eukaryotic cells. *In* Biomembranes. L. A. Manson, editor. Plenum Publishing Corp., New York. 2: 193–195.
20. Bonatti, S., and G. Blobel. 1979. Absence of a cleavable signal sequence in Sindbis virus glycoprotein PE$_2$. *J. Biol. Chem.* 254(24):12261–12264.
21. Bonatti, S., R. Cancedda, and G. Blobel. 1979. Membrane biogenesis: *in vitro* cleavage, core glycosylation and integration into microsomal membranes of Sindbis virus glycoproteins. *J. Cell Biol.* 80:219–224.
22. Borgese, D., G. Blobel, and D. D. Sabatini. 1973. *In vitro* exchange of ribosomal subunits between free and membrane-bound ribosomes. *J. Mol. Biol.* 74:415–438.
23. Borgese, N., and S. Gaetani. 1980. Site of synthesis of rat liver NADH-cytochrome-b_5 reductase, an integral membrane protein. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 112(2): 216–220.
24. Borgese, N., W. Mok, G. Kreibich, and D. D. Sabatini. 1974. Ribosome-membrane interaction: *in vitro* binding of ribosomes to microsomal membranes. *J. Mol. Biol.* 88: 559–580.
25. Botelho, L. H., D. E. Ryan, and W. Levin. 1979. Amino acid compositions and partial amino acid sequences of three highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biophenyls, phenobarbital or 3-methylcholanthrene. *J. Biol. Chem.* 254(13):5635–5640.
26. Brennan, S. O., and R. W. Carrell. 1978. A circulating variant of human proalbumin. *Nature (Lond.).* 274:908–909.
27. Bretscher, M. S. 1971. A major protein which spans the human erythrocyte membrane. *J. Mol. Biol.* 59:351–357.
28. Bretscher, M. S. 1973. Membrane structure: some general principles. *Science (Wash. D. C.).* 181:622–629.
29. Brown, M. S., and J. L. Goldstein. 1979. Receptor-mediated endocytosis: insights from the lipoprotein receptor system. *Proc. Natl. Acad. Sci. U. S. A.* 76:3330–3337.
30. Brunmer, J., H. Hauser, H. Braun, K. J. Wilson, H. Wacker, B. O'Neill, and G. Semenza. 1979. The mode of association of the enzyme complex sucrase-isomaltase with the intestinal brush border membrane. *J. Biol. Chem.* 254:1821–1828.
31. Chang, C. N., P. Model, and G. Blobel. 1979. Membrane biogenesis: cotranslational integration of the bacteriophage F1 coat protein into an *Escherichia coli* membrane fraction. *Proc. Natl. Acad. Sci. U. S. A.* 76:1251–1255.
32. Choppin, P. W., and A. Scheid. 1980. The role of viral glycoproteins in adsorption, penetration, and pathogenicity of viruses. *Rev. Infect. Dis.* 2(1):40–61.
33. Chua, N.-H., G. Blobel, P. Siekevitz, and G. E. Palade. 1973. Attachment of chloroplast polysomes to thylakoid membranes in *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. U. S. A.* 70(5):1554–1558.
34. Chua, N.-H., and G. W. Schmidt. 1978. Posttranslational transport into intact chloroplasts of a precursor to the small subunit of ribulose 1,5-biphosphate carboxylase. *Proc. Natl. Acad. Sci. U. S. A.* 75(12):6110–6114.
35. Chua, N.-H., and G. W. Schmidt. 1979. Transport of proteins into mitochondria and chloroplasts. *J. Cell Biol.* 81:461–483.
36. Chyn, T. L., A. N. Martonosi, T. Morimoto, and D. D. Sabatini. 1979. *In vitro* synthesis of the Ca^{++}-transport ATPase by ribosomes bound to sarcoplasmic reticulum membranes. *Proc. Natl. Acad. Sci. U. S. A.* 76:1241–1245.
37. Clegg, C., and I. Kennedy. 1975. Translation of Semliki-Forest-virus intracellular 26S RNA. Characterization of the products synthesized *in vitro*. *Eur. J. Biochem.* 53:175–185.
38. Conboy, J. G., F. Kalousek, and L. E. Rosenberg. 1979. *In vitro* synthesis of a putative precursor of mitochondrial ornithine transcarbarmoylase. *Proc. Natl. Acad. Sci. U. S. A.* 76(11):5724–5727.
39. Cote, C., Solioz, M., and Schatz, G. 1979. Biogenesis of the cytochrome bc_1 complex of yeast mitochondria. *J. Biol. Chem.* 254(5):1437–1439.
40. Cotmore, S. F., H. Furthmayr, and V. T. Marchesi. 1977. Immunochemical evidence for the transmembrane orientation of glycophorin A. Localization of ferritin-antibody conjugates in intact cells. *J. Mol. Biol.* 113:539–553.
41. Czernilofsky, A. P., A. D. Levinson, H. E. Varmus, J. M. Bishop, E. Tischer, and H. M. Goodman. 1980. Nucleotide sequence of an avian sarcoma virus oncogene (SRC) and proposed amino acid sequence for gene product. *Nature (Lond.).* 287:198–203.
42. Date, T., J. M. Goodman, and W. Wickner. 1980. Precoat, the precursor of M13 coat protein, requires an electrochemical potential for membrane insertion. *Proc. Natl. Acad. Sci. U. S. A.* 77:4669–4673.
43. Date, T., C. Zwizinski, S. Ludmerer, and W. Wickner. 1980. Mechanisms of membrane assembly: effects of energy poisons on the conversion of soluble M13 coliphage procoat to membrane-bound coat proteins. *Proc. Natl. Acad. Sci. U. S. A.* 77(2):827–831.
44. Davis, B. D., and P.-C. Tai. 1980. The mechanism of protein secretion across membranes. *Nature (Lond.).* 283:433–438.
45. de Duve, C., and R. Wattiaux. 1966. Functions of lysosomes. *Annu. Rev. Physiol.* 28:435–492.
46. DePierre, J. W., and G. Dallner. 1975. Structural aspects of the membrane of the endoplasmic reticulum. *Biochim. Biophys. Acta.* 415:411–472.
47. Devillers-Thiery, A., T. Kindt, G. Scheele, and G. Blobel. 1975. Homology in amino terminal sequence of precursors to pancreatic secretory proteins. *Proc. Natl. Acad. Sci. U. S. A.* 72:5016–5020.
48. Dobberstein, B., G. Blobel, and N.-H. Chua. 1977. *In vitro* synthesis and processing of a putative precursor for the small subunit of ribulose-1,5-biphosphate carboxylase of *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. U. S. A.* 74(3):1082–1085.
49. Dobberstein, B., H. Garoff, G. Warren, and P. J. Robinson. 1979. Cell-free synthesis and membrane insertion of mouse H-2D^d histocompatibility antigen and β_2-microglobulin *Cell.* 17:759–769.
50. Dubois, G. C., E. Appella, R. Armstrong, W. Levin, A. Y. H. Lu, and D. M. Jerina. 1979. Hepatic microsomal epoxide hydrase. Chemical evidence for a single polypeptide chain. *J. Biol. Chem.* 254(14):6240–6243.
51. Early, P., J. Rogers, M. Davis, K. Calame, M. Bond, R. Wall, and L. Hood. 1980. Two

mRNA can be produced from a single immunoglobulin μ gene by alternative RNA processing pathways. *Cell.* 20:313–320.
52. Elder, K. T., J. M. Bye, J. J. Skehel, M. D. Waterfield, and A. E. Smith. 1979. *In vitro* synthesis, glycosylation, and membrane insertion of influenza virus haemagglutinin. *Virology.* 95:343–350.
53. Emr, S. D., M. N. Hall, and T. J. Silhavy. 1980. A mechanism of protein localization: the signal hypothesis and bacteria. *J. Cell Biol.* 86:701–711.
54. Emr, S. D., J. Hedgpeth, J.-M. Clement, T. J. Silhavy, and M. Hofnung. 1980. Sequence analysis of mutations that prevent export of λ receptor, an *Escherichia coli* outer membrane protein. *Nature (Lond.).* 285:82–85.
55. Erickson, A. H., and G. Blobel. 1979. Early events in the biosynthesis of the lysosomal enzyme cathepsin D. *J. Biol. Chem.* 254:11771–11774.
56. Eylar, E. H. 1965. On the biological role of glycoproteins. *J. Theor. Biol.* 10:89–113.
57. Fagerhol, M. K. 1969. Quantitative studies on the inherited variants of serum α_1-antitrypsin. *Scand. J. Clin. Lab. Invest.* 23:97–103.
58. Fischer, H. D., A. Gonzalez-Noriega, and W. S. Sly. 1980. β-Glucuronidase binding to human fibroblast membrane receptors. *J. Biol. Chem.* 255(11):5069–5074.
59. Frank, G., J. Brunner, H. Hauser, H. Wacker, G. Semenza, and H. Zuber. 1978. The hydrophobic anchor of small-intestinal sucrase-isomaltase N-terminal sequence of the isomaltase subunit. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 96:183–188.
60. Franke, W. W., D. J. Morré, B. Deumling, R. D. Cheetham, J. Kartenbeck, E.-D., Jarasch, and H.-W. Zentgraf. 1971. Synthesis and turnover of membrane proteins in rat liver: an examination of the membrane flow hypothesis. *Z. Naturforsch.* 26b:1031–1039.
61. Fraser, T. H., and B. J. Bruce. 1978. Chicken ovalbumin is synthesized and secreted by *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 75(12):5936–5940.
62. Friedmann, T., A. Esty, P. LaPorte, and P. Deininger. 1979. The nucleotide sequence and genome organization of the polyoma early region: extensive nucleotide and amino acid homology with SV40. *Cell.* 17:715–724.
63. Friend, D. S., and M. G. Farquhar. 1967. Functions of coated vesicles during protein absorption in the rat vas deferens. *J. Cell Biol.* 35:357–376.
64. Fujii-Kuriyama, Y., M. Negishi, R. Mikawa, and Y. Tashioro. 1979. Biosynthesis of cytochrome P-450 on membrane-bound ribosomes and the subsequent incorporation into rough and smooth microsomes in rat hepatocytes. *J. Cell Biol.* 81:510–519.
65. Fukuda, M. N., D. S. Papermaster, and P. A. Hargrave. 1979. Rhodopsin carbohydrate structure of small oligosaccharides attached at two sites near the NH_2 terminus. *J. Biol. Chem.* 254(17):8201–8207.
66. Garoff, H., A.-M., Frischauf, K. Simons, H. Lehrach, and H. Delius. 1980. Nucleotide sequence of cDNA coding for Semliki Forest virus membrane glycoproteins. *Nature (Lond.).* 288:236–241.
67. Garoff, H., K. Simons, and B. Dobberstein. 1978. Assembly of the Semliki Forest virus membrane glycoproteins in the membrane of the endoplasmic reticulum *in vitro*. *J. Mol. Biol.* 124:587–600.
68. Garoff, H., K. Simons, and O. Renkonen. 1974. Isolation and characterization of the membrane proteins of Semliki Forest virus. *Virology.* 61:493–504.
69. Garoff, H., and H. Söderlund. 1978. The amphiphilic membrane glycoproteins of Semliki Forest virus are attached to the lipid bilayer by their COOH-terminal ends. *J. Mol. Biol.* 124:535–549.
70. Gething, M. J., J. M. White, and M. D. Waterfield. 1978. Purification of the fusion protein of Sendai virus: analysis of the NH_2-terminal sequence generated during precursor activation. *Proc. Natl. Acad. Sci. U. S. A.* 75(6):2737–2740.
71. Gibson, R., R. Leavitt, S. Kornfeld, and S. Schlesinger. 1978. Synthesis and infectivity of vesicular stomatitis viruses containing nonglycosylated G protein. *Cell.* 13:671–679.
72. Gibson, R., S. Schlesinger, and S. Kornfeld. 1979. The nonglycosylated glycoprotein of vesicular stomatitis virus is temperature-sensitive and undergoes intracellular aggregation at elevated temperatures. *J. Biol. Chem.* 254(9):3600–3607.
73. Goldman, B. M., and G. Blobel. 1978. Biogenesis of peroxysomes: intracellular site of synthesis of catalase and uricase. *Proc. Natl. Acad. Sci. U. S. A.* 75(10):5066–5070.
74. Goldstein, J. L., R. G. W. Anderson, and M. S. Brown. 1979. Coated pits, coated vesicles, and receptor-mediated endocytosis. *Nature (Lond.).* 279:679–685.
75. Gonzalez, F. J., and C. B. Kasper. 1980. Phenobarbital induction of NADPH-cytochrome *c* (P-450) oxidoreductase messenger ribonucleic acid. *Biochemistry.* 19:1790–1796.
76. Gonzalez, F. J., and C. B. Kasper. 1980. In vitro translation of epoxide hydratase messenger RNA. *Biochem. Biophys. Res. Commun.* 93:1254–1258.
77. Gonzalez-Noriega, A., J. H. Grubb, V. Talkad, and W. S. Sly. 1980. Chloroquine inhibits lysosomal enzyme pinocytosis and enhances lysosomal enzyme secretion by impairing receptor recycling. *J. Cell Biol.* 85:839–852.
78. Green, R. F., H. K. Meiss, and E. Rodriguez-Boulan. 1981. Glycosylation does not determine segregation of viral envelope proteins in the plasma membrane of epithelial cells. *J. Cell Biol.* 89:230–239.
79. Greenway, D. C., and D. H. MacLennan. 1978. Assembly of the sarcoplasmic reticulum. Synthesis of calsequestrin and the Ca^{2+} and Mg^{2+}-adenosine triphosphatase on membrane-bound polyribosomes. *Can. J. Biochem.* 56:452–456.
80. Grossman, A., S. Bartlett, and N.-H. Chua. 1980. Energy-dependent uptake of cytoplasmically synthesized polypeptides by chloroplasts. *Nature (Lond.).* 285:625–628.
81. Habener, J. F., R. Maunus, P. C. Dee, and J. T. Potts, Jr. 1980. Early events in the cellular formation of proparathyroid hormone. *J. Cell Biol.* 85:292–298.
82. Hallermayer, G., R. Zimmerman, and W. Neupert. 1977. Kinetic studies on the transport of cytoplasmically synthesized proteins into the mitochondria in intact cells of *Neurospora crassa*. *Eur. J. Biochem.* 81:523–532.
83. Hargrave, P. A. 1977. The amino-terminal tryptic peptide of bovine rhodopsin. A glycopeptide containing two sites of oligosaccharide attachment. *Biochim. Biophys. Acta* 492:83–94.
84. Harmey, M. A., G. Hallermayer, H. Korb, and W. Neupert. 1977. Transport of cytoplasmically synthesized proteins into the mitochodnria in a cell-free system from *Neurospora crassa*. *Eur. J. Biochem.* 81:533–544.
85. Harmey, M., and W. Neupert. 1979. Biosynthesis of mitochondrial synthetase in *Neurospora crassa*. *FEBS (Fed. Eur. Biochem. Soc.) Letts.* 108(2):385–389.
86. Hasilik, A., and E. Neufeld. 1980. Biosynthesis of lysosomal enzymes in fibroblasts—synthesis as precursors of higher molecular weight. *J. Biol. Chem.* 255:4937–4945.
87. Hasilik, A., and E. Neufeld. 1980. Biosynthesis of lysosomal enzymes in fibroblasts—phosphorylation of mannose residues. *J. Biol. Chem.* 255:4946–4950.
88. Hauri, H.-P., A. Quaroni, and K. J. Isselbacher. 1979. Biogenesis of intestine plasma membrane: posttranslational route and cleavage of sucrase-isomaltase. *Proc. Natl. Acad. Sci. U. S. A.* 76(10):5183–5186.
89. Hickman, S., A. Kulczycki, Jr., R. G. Lynch, and S. Kornfeld. 1977. Studies on the mechanism of tunicamycin inhibition of IgA and IgE secretion by plasma cells. *J. Biol. Chem.* 252(12):4402–4408.
90. Hickman, S., L. J. Shapiro, and E. F. Neufeld. 1974. A recognition marker for uptake of a lysosomal enzyme by cultured fibroblasts. *Biochem. Biophys. Res. Commun.* 51:55–61.
91. Highfield, P. E., and R. J. Ellis. 1978. Synthesis and transport of the small subunit of chloroplast ribulose biophosphate carboxylase. *Nature (Lond.).* 271:420–424.
92. Hutchinson, M. A., T. Hunter, and W. Eckhart. 1978. Characterization of T antigens in polyoma-infected and transformed cells. *Cell.* 15:65–77.
93. Inouye, H., and J. Beckwith. 1977. Synthesis and processing of an *Escherichia coli* alkaline phosphatase precursor *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 74(4):1440–1444.
94. Inouye, M., and S. Halegoua. 1979. Secretion and membrane localization of proteins in *Escherichia coli*. *Crit. Rev. Biochem.* 7:339–371.
95. Inouye, S., S. Wang, J. Sekizawa, S. Halegoua, and M. Inouye. 1977. Amino acid sequence for the peptide extension on the prolipoprotein of the *Escherichia coli* outer membrane. *Proc. Natl. Acad. Sci. U. S. A.* 74(3):1004–1008.
96. Irving, R. A., F. Toneguzzo, S. H. Rhee, T. Hofmann, and H. P. Ghosh. 1979. Synthesis and assembly of membrane glycoproteins: presence of leader peptide in nonglycosylated precursor of membrane glycoprotein of vesicular stomatitis virus. *Proc. Natl. Acad. Sci. U. S. A.* 76(2):570–574.
97. Ito, Y., J. R. Brocklehurst, and R. Dulbecco. 1977. Virus-specific proteins in the plasma membrane of cells lytically infected on transformed by polyoma virus. *Proc. Natl. Acad. Sci. U. S. A.* 74(10):4666–4670.
98. Ito, K., T. Date, and W. Wickner. 1980. Synthesis, assembly into the cytoplasmic membrane, and proteolytic processing of the precursor of coliphage M13 coat protein. *J. Biol. Chem.* 255:2123–2130.
99. Ito, K., G. Mandel, and W. Wickner. 1979. Soluble precursor of an integral membrane protein: synthesis of precoat protein in *Escherichia coli* infected with bacteriophage M13. *Proc. Natl. Acad. Sci. U. S. A.* 76(3):1199–1203.
100. Jackson, R. C., and G. Blobel. 1977. Posttranslational cleavage of presecretory proteins with an extract of rough microsomes from dog pancreas containing signal peptidase activity. *Proc. Natl. Acad. Sci. U. S. A.* 74(12):5598–5602.
101. Jackson, R. C., and G. Blobel. 1980. Posttranslational processing of full-length presecretory proteins with canine pancreatic signal peptidase. *Ann. N. Y. Acad. Sci.* 343:391–404.
102. Jackson, R. C., P. Walter, and G. Blobel. 1980. A cytoplasmically disposed sulfhydryl group required for the translocation of nascent presecretory proteins across the membrane of the rough endoplasmic reticulum. *Nature (Lond.)* 284:174–176.
103. Jamieson, J. D., and G. E. Palade. 1971. Synthesis, intracellular transport, and discharge of secretory proteins in stimulated pancreatic exocrine cells. *J. Cell Biol.* 50:135–158.
104. Judah, J. D., and P. S. Quinn. 1978. Calcium ion-dependent vesicle fusion in the conversion of proalbumin to albumin. *Nature (Lond.).* 271:384–385.
105. Kaplan, A., D. T. Achord, and W. S. Sly. 1977. Phosphohexosyl components of a lysosomal enzyme are recognized by pinocytosis receptors on human fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 74(5):2026–2030.
106. Katz, F. N., J. E. Rothman, D. M. Knipe, and H. F. Lodish. 1977. Membrane assembly: Synthesis and intracellular processing of the vesicular stomatitis viral glycoprotein. *J. Supramol. Struct.* 7:353–370.
107. Katz, F. N., J. E. Rothman, V. R. Lingappa, G. Blobel, and H. F. Lodish. 1977. Membrane assembly *in vitro*: synthesis, glycosylation and asymmetric insertion of a transmembrane protein. *Proc. Natl. Acad. Sci. U. S. A.* 74:3278–3282.
108. Kehry, M., S. Ewald, R. Douglas, C. Sibley, W. Raschke, D. Fambrough, and L. Hood. 1980. The immunoglobulin μ chains of membrane-bound and secreted IgM molecules differ in their C-terminal segments. *Cell.* 21:393–406.
109. Kemper, B., J. F. Habener, R. C. Mulligan, J. T. Potts, Jr., and A. Rich. 1974. Preproparathyroid hormone: a direct translation product of a parathyroid messenger RNA. *Proc. Natl. Acad. Sci. U. S. A.* 71:3731–3735.
110. Korb, H., and W. Neupert. 1978. Biogenesis of cytochrome *c* in *Neurospora crassa*. *Eur. J. Biochem.* 91:609–620.
111. Koshland, D., and D. Botstein. 1980. Secretion of beta-lactamase requires the carboxy end of the protein. *Cell.* 20:749–760.
112. Kreibich, G., S. Bar-Nun, M. Czako-Graham, W. Mok, E. Nack, Y. Okada, M. G. Rosenfeld, and D. D. Sabatini. 1980. The role of free and membrane bound polysomes in organelle biogenesis. *In* Biological Chemistry of Organelle Formation. *Proceedings of the Mosbach Colloquia.* Th. Bucher, W. Sebald, and H. Weiss, editors. Singer Verlag, Berlin. 147–163.
113. Kreibich, G., M. Czako-Graham, R. Grebenau, W. Mok, E. Rodriguez-Boulan, and D. D. Sabatini. 1978. Characterization of the ribosomal binding site in rat liver rough microsomes: ribophorins I and II, two integral membrane proteins related to ribosome binding. *J. Supramol. Struct.* 9:279–292.
114. Kreibich, G., M. Czako-Graham, R. C. Grebenau, and D. D. Sabatini. 1980. Functional and structural characteristics of endoplasmic reticulum proteins associated with ribosome binding sites. *Ann. N. Y. Acad. Sci.* 343:17–33.
115. Kreibich, G., C. M. Freienstein, B. N. Pereyra, B. L. Ulrich, and D. D. Sabatini. 1978. Proteins of rough microsomal membranes related to ribosome binding. II. Cross-linking of bound ribosomes to specific membrane proteins exposed at the binding sites. *J. Cell Biol.* 77:488–506.
116. Kreibich, G., and D. D. Sabatini. 1974. Selective release of content from microsomal vesicles without membrane disassembly. II. Electrophoretic and immunological characterization of microsomal subfractions. *J. Cell Biol.* 61:789–807.
117. Kreibich, G., B. L. Ulrich, and D. D. Sabatini. 1978. Proteins of rough microsomal membranes related to ribosome binding. I. Identification of ribophorins I and II, membrane proteins characteristic of rough microsomes. *J. Cell Biol.* 77:464–487.
117a. Kreil, G. 1981. Transfer of proteins across membranes. *Annu. Rev. Biochem.* 50:317–348.
118. Kuriyama, Y., and D. J. Luck. 1973. Membrane-associated ribosomes in mitochondria of *Neurospora crassa*. *J. Cell Biol.* 59:776–784.
119. Lampen, J. O. 1974. Movement of extracellular enzymes across cell membranes. *In* Transport at the cellular level. *Symp. Soc. Exp. Biol.* 28:351–374.
120. Lewin, A. S., I. Gregor, T. L. Mason, N. Nelson, and G. Schatz. 1980. Cytoplasmically made subunits of yeast mitochondrial F_1-ATPase and cytochrome *c* oxidase are synthesized as individual precursors, not as polyproteins. *Proc. Natl. Acad. Sci. U. S. A.* 77: 3998–4002.
121. Lewis, J. A., and D. D. Sabatini. 1977. Proteins of rat liver free and membrane-bound ribosomes. Modification of two large subunit proteins by a factor detached from ribosomes at high ionic strength. *Biochim. Biophys. Acta.* 478:331–349.
122. Lin, J. J. C., H. Kanzawa, and H. C. Wu. 1980. Purification and characterization of the outer membrane lipoprotein from an *Escherichia coli* mutant altered in the signal sequence of prolipoprotein. *J. Biol. Chem.* 255:1160–1163.
123. Lingappa, V. R., B. A. Cunningham, S. M. Jazwinski, T. P. Hopp, G. Blobel, and G. M. Edelman. 1979. Cell-free synthesis and segregation of β_2-microglobulin. *Proc. Natl. Acad. Sci. U. S. A.* 76:3651–3655.
124. Lingappa, V. R., F. N. Katz, H. F. Lodish, and G. Blobel. 1978. A signal sequence for the insertion of a transmembrane glycoprotein. Similarities to the signals of secretory proteins in primary structure and function. *J. Biol. Chem.* 253:8667–8670.
125. Lingappa, V. R., J. R. Lingappa, and G. Blobel. 1979. Chicken ovalbumin contains an internal signal sequence. *Nature (Lond.).* 281:117–121.
126. Lingappa, V. R., D. Shields, S. L. C. Woo, and G. Blobel. 1978. Nascent chicken

ovalbumin contains the functional equivalent of a signal sequence. *J. Cell Biol.* 79:567–572.
127. Lodish, H. F., W. A. Braell, A. L. Schwartz, A. L. Schwartz, G. J. A. M., Strous, and A. Zilberstein. 1981. Synthesis and assembly of membrane organelles proteins. *Int. Rev. Cytol.* 241–307.
128. Maccecchini, M. L., Y. Rudin, G. Blobel, and G. Schatz. 1979. Import of proteins into mitochondria: precursor forms of the extramitochondrially made F_1-ATPase subunits in yeast. *Proc. Natl. Sci. U. S. A.* 76:343–347.
129. Maccecchini, M.-L., Y. Rudin, and G. Schatz. 1979. Transport of proteins across the mitochondrial outer membrane. *J. Biol. Chem.* 254:7468–7471.
130. Madjar, J.-J., M. Arpin, M. Buisson, and J.-P. Reboud. 1979. Spot position of rat liver ribosomal proteins by four different two dimensional electrophoresis in polyacrylamide gel. *Mol. Gen. Genet.* 171:121–134.
131. Malkin, L. I., and A. Rich. 1967. Partial resistance of nascent polypeptide chains to proteolytic digestion due to ribosomal shielding. *J. Mol. Biol.* 26:329–346.
132. Margoliash, E., W. M. Fitch, and R. E. Dickerson. 1971. Molecular expression of evolutionary phenomena in the primary and tertiary structures of cytochrome *c*. *In* Molecular Evolution. II. Biochemical Evolution and the Origin of Life. E. Schoffeniels, editor. 52–95.
133. Matsuura, M., M. Arpin, C. Hannum, E. Margoliash, D. D. Sabatini, and T. Morimoto. 1981. In vitro synthesis and posttranslational uptake of cytochrome *c* into isolated mitochondria. Role of a specific addressing signal in the apocytochrome. *Proc. Natl. Acad. Sci. U. S. A.* 78:4368–4372.
134. Matsuura, S., Y. Fujii-Kuriyama, and Y. Tashiro. 1978. Immunoelectron microscope localization of cytochrome P-450 on microsomes and other membrane structures of rat hepatocytes. *J. Cell Biol.* 78:503–519.
135. Meek, R. L., K. A. Walsh, and R. Palmiter. 1980. Ovalbumin contains an NH_2-terminal functional equivalent of a signal sequence. *Fed Proc.* 39:1867. (Abstr.).
136. Meyer, D. I., and B. Dobberstein. 1980. A membrane component essential for vectorial translocation of nascent proteins across the endoplasmic reticulum: requirements for its extraction and reassociation with the membrane. *J. Cell Biol.* 87:498–502.
137. Meyer, D. I., and B. Dobberstein. 1980. Identification and characterization of a membrane component essential for the translocation of nascent proteins across the membrane of the endoplasmic reticulum. *J. Cell Biol.* 87:503–508.
138. Milstein, C., G. G. Brownlee, T. M. Harrison, and M. B. Mathews. 1972. A possible precursor of immunoglobulin light chains. *Nat. New Biol.* 239:117–120.
139. Minjou, W., G. Threlfall, M. Verhoeyen, R. Devos, E. Saman, R. Fang, D. Huylebroeck, W. Fiers, C. Barber, N. Carey, and S. Emtage. 1980. Complete structure of hemagglutinin gene from the human influenza A/Victoria/3/75 (H3N2) strain as determined from clones DNA. *Cell.* 19:683–696.
140. Moreno, F., A. V. Fowler, M. Hall, T. J. Silhavy, I. Zabin, and M. Schwartz. 1980. A signal sequence is not sufficient to lead β-galactosidase out of the cytoplasm. *Nature (Lond.)* 286:356–359.
141. Morri, M., S. M. Morris, Jr., and P. P. Cohen. 1979. Cell-free translation and thyroxine induction of carbamyl phosphate synthetase I messenger RNA in tadpole liver. *Proc. Natl. Acad. Sci. U. S. A.* 76:3179–3183.
142. Mosmann, T. R., and A. R. Williamson. 1980. Structural mutations in a mouse immunoglobulin light chain resulting in failure to be secreted. *Cell.* 20:283–292.
143. Movva, N. R., K. Nakamura, and M. Inouye. 1980. Amino acid sequence of the signal peptide of ompA protein, a major outer membrane protein of *Escherichia coli*. *J. Biol. Chem.* 255:27–29.
144. Natori, T., N. Tanigaki, E. Appella, and D. Pressman. 1975. Amino acid composition and physicochemical properties of mouse β_2-microglobulin. *Biochem. Biophys. Res. Commun.* 65:611–617.
145. Natowicz, M. R., M. M.-Y., Chi, O. H. Lowry, and W. S. Sly. 1979. Enzymatic identification of mannose-6-phosphate on the recognition marker for receptor-mediated pinocytosis of β-glucuronidase by human fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 76: 4322–4326.
146. Negishi, M., T. Sawamura, T. Morimoto, and Y. Tashiro. 1975. Localization of nascent NADPH-cytochrome *c* reductase in rat liver microsomes. *Biochim. Biophys. Acta.* 381: 215–220.
147. Negishi, M., Y. Fujii-Kuriyama, Y. Tashiro, and Y. Imai. 1976. Site of biosynthesis of cytochrome P-450 in hepatocytes of phenobarbital treated rats. *Biochem. Biophys. Res. Commun.* 71:1153–1160.
148. Nelson, N., and G. Schatz. 1979. Energy-dependent processing of cytoplasmically made precursors to mitochondrial proteins. *Proc. Natl. Acad. Sci. U. S. A.* 76:4365–4369.
149. Neurath, H., and K. A. Walsh. 1976. Role of proteolytic enzymes in biological regulation. *Proc. Natl. Acad. Sci. U. S. A.* 73:3825–3832.
150. Novikoff, A. B. 1976. The endoplasmic reticulum: a cytochemist's view (a review) *Proc. Natl. Acad. Sci. U. S. A.* 73:2781–2787.
151. Okada, Y., D. D. Sabatini, and G. Kreibich. 1979. Site of synthesis of rat liver cytochrome b_5 and NADPH-cytochrome P-450 reductase. *J. Cell Biol.* 83(2, Pt. 2):437*a*, (Abstr.).
152. Oxender, D. L., J. J. Anderson, C. J. Daniels, R. Landick, R. P. Gunsalus, G. Zurawski, and C. Yanofsky. 1980. Amino-terminal sequence and processing of the precursor of the leucine-specific binding protein, and evidence for conformational differences between the precursor and the mature form. *Proc. Natl. Acad. Sci. U. S. A.* 77:2005–2009.
153. Ozols, J., and C. Gerard. 1977. Primary structure of the membranous segment of cytochrome b_5. *Proc. Natl. Acad. Sci. U. S. A.* 74:3725–3729.
154. Palade, G. E. 1966. Structure and function at the cellular level. *JAMA (J. Am. Med. Assoc.).* 198:815–825.
155. Palade, G. E. 1975. Intracellular aspects of the process of protein synthesis. *Science (Wash. D. C.).* 189:347–358.
156. Palmiter, R. D., J. Gagnon, L. H. Ericsson, and K. A. Walsh. 1977. Precursor of egg white lysosyme. Amino acid sequence of an NH_2-terminal extension. *J. Biol. Chem.* 252: 6386–6393.
157. Palmiter, R. D., J. Gagnon, and K. A. Walsh. 1978. Ovalbumin: a secreted protein without a transient hydrophobic leader sequence. *Proc. Natl. Acad. Sci. U. S. A.* 75:94–98.
158. Patzelt, C., A. D. Labrecque, J. R. Duguid, R. J. Carroll, P. S. Keim, R. L. Heinrikson, and D. F. Steiner. 1978. Detection and kinetic behavior of preproinsulin in pancreatic islets. *Proc. Natl. Sci. U. S. A.* 75:1260–1264.
159. Pearse, B. M. F. 1976. Clathrin: a unique protein associated with intracellular transfer of membrane by coated vesicles. *Proc. Natl. Acad. Sci. U. S. A.* 73:1255–1259.
160. Peterson, P. A., L. Rask, and J. B. Lindblom. 1974. Highly purified papain-solubilized HL-A antigens contain β_2-microglobulin. *Proc. Natl. Acad. Sci. U. S. A.* 71:35–39.
161. Ploegh, H. L., L. E. Cannon, and J. L. Strominger. 1979. Cell-free translation of the mRNA's for the heavy and light chains of HLA-A and HLA-B antigens. *Proc. Natl. Acad. Sci. U. S. A.* 76:2273–2277.
162. Ploegh, H. L., H. T. Orr, and J. L. Strominger. 1980. Molecular cloning of a human histocompatibility antigen cDNA fragment. *Proc. Natl. Sci. U. S. A.* 77:6081–6085.
163. Podack, E. R., G. Biesecker, and H. J. Muller-Eberhard. 1979. Membrane attack complex of complement: generation of high-affinity phospholipid binding sites by fusion of five hydrophilic plasma proteins. *Proc. Natl. Sci. U. S. A.* 76:879–901.
164. Popov, D., L. Alterman, D. D. Sabatini, and G. Kreibich. 1978. In vitro synthesis of β-glucuronidase by rat liver and preputial gland membrane bound ribosomes. *J. Cell Biol.* 79(2, Pt. 2): 364*a* (Abstr.).
165. Poyton, R. O., and E. McKemmie. 1979. Post-translational processing and transport of polyprotein precursor to subunits IV and VII of yeast cytochrome *c* oxidase. *J. Biol. Chem.* 254:6772–6780.
166. Prehn, S., A. Tsamaloukas, and T. A. Rapoport. 1980. Demonstration of specific receptors of the rough endoplasmic membrane for the signal sequence of carp preproinsulin. *Eur. J. Biochem.* 107:185–195.
167. Rachubinski, R. A., D. P. S. Verma, and J. J. M. Bergeron. 1980. Synthesis of rat liver microsomal cytochrome b_5 by free ribosomes. *J. Cell Biol.* 84:705–716.
168. Randall, L. L., S. J. S. Hardy, and L.-G. Josefsson. 1978. Precursors of three exported proteins in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 75:1209–1212.
169. Redman, C. M., and D. D. Sabatini. 1966. Vectorial discharge of peptides released by puromycin from attached ribosomes. *Proc. Natl. Acad. Sci. U. S. A.* 56:608–615.
170. Redman, C. M., P. Siekevitz, and G. E. Palade. 1966. Synthesis and transfer of amylase in pigeon pancreatic microsomes. *J. Biol. Chem.* 241:1150–1158.
171. Ries, G., E. Hundt, and B. Kadenbach. 1978. Immunoprecipitation of a cytoplasmic precursor of rat-liver cytochrome oxidase. *Eur. J. Biochem.* 91:179–191.
172. Rodriguez-Boulan, E., and D. D. Sabatini. 1978. Asymmetric budding of viruses in epithelial monolayers: a model system for study of epithelial polarity. *Proc. Natl. Acad. Sci. U. S. A.* 75:5071–5075.
173. Rodriguez-Boulan, E., D. D. Sabatini, B. N. Pereyra, and G. Kreibich. 1978. Spatial orientation of glycoproteins in membranes of rat liver rough microsomes. II. Transmembrane disposition and characterization of glycoproteins. *J. Cell Biol.* 78:894–909.
174. Rogers, J., P. Early, C. Carter, K. Calame, M. Bond, L. Hood, and R. Wall. 1980. Two mRNAs with different 3' ends encode membrane-bound and secreted forms of immunoglobulin μ chain. *Cell.* 22:303–312.
175. Rose, J. K., W. J. Welch, B. M. Sefton, F. S. Esch, and N. C. Ling. 1980. Vesicular stomatitis virus glycoprotein is anchored in the viral membrane by a hydrophobic domain near the COOH terminus. *Proc. Natl. Acad. Sci. U. S. A.* 77:3884–3888.
176. Rosenfeld, M., D. D. Sabatini, E. Sabban, K. Kato, and G. Kreibich. 1980. Synthesis and maturation of lysosomal enzymes. *J. Cell Biol.* 87(2, Pt. 2): 308*a* (Abstr.).
177. Roth, M. G., J. P. Fitzpatrick, and R. W. Compans. 1979. Polarity of influenza and vesicular stomatitis virus in MDCK cells; lack of a requirement for glycosylation of viral glycoproteins. *Proc. Natl. Acad. Sci. U. S. A.* 76:6430–6434.
178. Roth, T. F., and K. R. Porter. 1964. Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti* L. *J. Cell Biol.* 20:313–332.
179. Rothman, J. E., H. Bursztyn-Pettegrew, and R. E. Fine. 1980. Transport of the membrane glycoprotein of vesicular stomatitis virus to the cell surface in two stages by clathrin-coated vesicles. *J. Cell Biol.* 86:162–171.
180. Rothman, J. E., and R. E. Fine. 1980. Coated vesicles transport newly synthesized membrane glycoproteins from endoplasmic reticulum to plasma membrane in two successive stages. *Proc. Natl. Acad. Sci. U. S. A.* 77:780–784.
181. Rothman, J. E., and J. Lenard. 1977. Membrane asymmetry. The nature of membrane asymmetry provides clues to the puzzle of how membranes are assembled. *Science (Wash. D. C.).* 195:743–753.
182. Rothman, J. E., and H. F. Lodish. 1977. Synchronized transmembrane insertion and glycosylation of a nascent membrane protein. *Nature (Lond.).* 269:775–780.
183. Rothman, S. S. 1980. Passage of proteins through membranes—old assumptions and new perspectives. *Am. J. Physiol.* 238:G391–G402.
184. Russell, J. H., and D. M. Geller. 1975. The structure of rat proalbumin. *J. Biol. Chem.* 250:3409–3413.
185. Sabatini, D. D., and G. Blobel. 1970. Controlled proteolysis of nascent polypeptides in rat liver cell fractions. II. Location of the polypeptides in rough microsomes. *J. Cell Biol.* 45:146–157.
186. Sabatini, D. D., G. Blobel, Y. Nonomura, and M. R. Adelman. 1971. Ribosome-membrane interaction: structural aspects and functional implications. *Adv. Cytopharmacol.* 1:119–129.
187. Sabatini, D. D., D. Borgese, M. Adelman, G. Kreibich, and G. Blobel. 1972. Studies on the membrane associated protein synthesis apparatus of eukaryotic cells. *In* RNA Viruses/Ribosomes. H. Bloemendal, E. J. Jaspers, A. Van Kammer, and R. J. Planta, editors. *Fed. Eur. Biochem. Soc. Symp. (Berlin).* 27:147–172.
188. Sabatini, D. D., and G. Kreibich. 1976. Functional specialization of membrane-bound ribosomes in eukaryotic cells. *In* The Enzymes of Biological Membranes, Vol. 2. A. Martonosi, editor. Plenum Publishing Co., New York. 531–579.
189. Sabatini, D. D., Y. Tashiro, and G. E. Palade. 1966. On the attachment of ribosomes to microsomal membranes. *J. Mol. Biol.* 19:503–524.
190. Sabban, E., D. D. Sabatini, M. Adesnik, and B. Marchesi. 1979. Biosynthesis of murine erythrocyte membrane protein band 3. *J. Cell Biol.* 83(2, Pt. 2):437*a* (Abstr.).
191. Sando, G. N., and E. F. Neufeld. 1977. Recognition and receptor-mediated uptake of a lysosomal enzyme, α-l-iduronidase by cultured human fibroblasts. *Cell.* 12:619–627.
192. Schatz, G. 1979. How mitochondria import proteins from the cytoplasm. 7th FEBS-Ferdinand Springer Lecture. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 103:201–211.
193. Schatz, G., and T. L. Mason. 1974. The biosynthesis of mitochondrial proteins. *Annu. Rev. Biochem.* 43:51–87.
194. Schechter, I. 1973. Biologically and chemically pure mRNA coding for mouse immunoglobulin L-chain prepared with the aid of antibodies and immobilized oligothymidine. *Proc. Natl. Acad. Sci. U. S. A.* 70:2256–2260.
195. Schechter, I., Y. Burstein, R. Zemell, E. Ziv, F. Kantor, and D. Papermaster. 1979. Messenger RNA of opsin from bovine retina: isolation and partial sequence of the *in vitro* translation product. *Proc. Natl. Acad. Sci. U. S. A.* 76:2654–2658.
195a. Scheele, G., B. Dobberstein, and G. Blobel. 1978. Transfer of proteins across membranes. Biosynthesis *in vitro* of pretrypsinogen and trypsinogen by cell fractions of canine pancreas. *Eur. J. Biochem.* 82:593–599.
195b. Scheele, G., R. Jacoby, and T. Carne. 1980. Mechanism of compartmentation of secretory proteins: transport of exocrine pancreatic proteins across the microsomal membrane. *J. Cell Biol.* 87:611–628.
196. Schmidt, G. W., A. Devillers-Thiery, H. Desruisseaux, G. Blobel, and N.-H. Chua. 1979. NH_2-terminal amino acid sequences of precursor and mature forms of the ribulose-1,5-bisphosphate carboxylase small subunit from *Chlamydomonas reinhardtii*. *J. Cell Biol.* 83:615–622.
197. Schmidt, M. F. G., and M. J. Schlesinger. 1980. Relation of fatty acid attachment to the translation and maturation of vesicular stomatitis and Sindbis virus membrane glycoproteins. *J. Biol. Chem.* 255:3334–3339.
198. Silhavy, T. J., P. Bassford, and J. Beckwith. 1979. A genetic approach to the study of protein localization in *Escherichia coli*. *In* Bacterial Outer Membranes: Biogenesis and

Function. M. Inouye, editor. John Wiley & Sons, New York. 203–254.
199. Silhavy, T. J., H. A. Shuman, J. R. Beckwith, and M. Schwartz. 1977. Use of gene fusions to study other membrane protein localization in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 74:5411–5415.
200. Sevarino, K. A., and R. O. Poyton. 1980. Mitochondrial membrane biogenesis: indentification of a precursor to yeast cytochrome *c* oxidase subunit II, and integral polypeptide. *Proc. Natl. Acad. Sci. U. S. A.* 77:142–146.
201. Sherman, J. M., D. D. Sabatini, and T. Morimoto. 1980. Studies on the biosynthesis of the polypeptide subunits of the Na,K-ATPase of kidney cells. *J. Cell Biol.* 87(2, Pt. 2): 307*a* (Abstr.).
202. Shimada, A., and S. G. Nathenson. 1969. Murine histocompatibility-2 (H-2) alloantigens. Purification and some chemical properties of soluble products from $H\text{-}2^b$ and $H\text{-}2^d$ genotypes released by papain digestion of membrane fractions. *Biochemistry.* 8(10):4048–4062.
203. Shore, G. C., P. Carignan, and Y. Raymond. 1979. *In vitro* synthesis of a putative precursor to the mitochondrial enzyme, carbamyl phosphate synthetase. *J. Biol. Chem.* 254:3141–3144.
204. Simmons, D. T., and J. H. Strauss. 1974. Translation of Sindbis virus 26S RNA and 49S RNA in lysates of rabbit reticulocytes. *J. Mol. Biol.* 86:397–409.
205. Simons, K., S. Keranen, and L. Kaariainen. 1973. Identification of a precursor for one of the Semliki Forest virus membrane proteins. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 29: 87–91.
206. Singer, P. A., and A. R. Williamson. 1980. Different species of messenger RNA encode receptor and secretory IgM chains differing at their carboxyl termini. *Nature (Lond.).* 285:294–299.
207. Singer, S. J. 1977. Thermodynamics, the structure of integral membrane proteins and transport. *J. Supramol. Struct.* 6:313–323.
208. Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science (Wash. D. C.)* 175:720–731.
209. Sly, W., and P. Stahl. 1978. Receptor mediated uptake of lysosomal enzymes. *In* Transport of Macromolecules in Cellular System. Life science research report II. S. Silverstein, editor. Dahlem Konferenzen, Berlin. 229–245.
210. Smart, J. E., and Y. Ito. 1978. Three species of polyoma virus tumor antigens share common peptides probably near the amino terminal of the proteins. *Cell.* 15:1427–1437.
211. Smith, W. P., P.-C. Tai, and B. D. Davis. 1978. Nascent peptide as sole attachment of polysomes to membranes in bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 75:814–817.
212. Smith, W. P., P.-C. Tai, and B. D. Davis. 1979. Extracellular labeling of growing secreted polypeptide chains in *Bacillus subtilis* with diazoiodosulfanilic acid. *Biochemistry.* 18:198–202.
213. Smith, W. P., P.-C., Tai, J. R. Murphy, and B. D. Davis. 1980. Precursor in cotranslational secretion of diphtheria toxin. *J. Bacteriol.* 141:184–189.
214. Smith, W. P., P.-C. Tai, R. C. Thompson, and B. D. Davis. 1977. Extracellular labeling of nascent polypeptides traversing the membrane of *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 74:2830–2834.
215. Soeda, E., J. R. Arrand, N. Smolar, and B. E. Griffinn. 1979. Sequence from early region of polyoma virus DNA containing viral replication origin and encoding small, middle and (part of) large T antigens. *Cell.* 17:357–370.
216. Steck, T. L. 1978. The band 3 protein in the human red cell membrane. *J. Supramol. Struct.* 8:311–324.
217. Steiner, D. F., W. Kemmler, J. L. Clark, P. E. Oyer, and A. H. Rubenstein. 1972. The biosynthesis of insulin. *In* Handbook of Physiology. Endocrinology I. D. F. Steiner and N. Freinkel, editors. Williams and Wilkins, Baltimore. 175–198.
218. Steiner, D. F., P. S. Quinn, S. J. Chan, J. Marsh, and H. S. Tager. 1980. Processing mechanisms in the biosynthesis of protein. *Ann. N. Y. Acad. Sci.* 343:1–16.
219. Struck, D. K., and W. J. Lennarz. 1980. The function of saccharide-lipids in synthesis of glycoprotein. *In* The Biochemistry of Glycoproteins and Proteoglycans. W. J. Lennarz, editor. Plenum Press, New York. 35–84.
220. Struck, D. K., P. B. Siuta, M. D. Lane, and W. J. Lane. 1978. Effect of tunicamycin on the secretion of serum proteins by primary cultures of rat and chick hepatocytes. *J. Biol. Chem.* 253:5332–5337.
221. Sugimoto, K., H. Sugisaki, T. Okamoto, and M. Takanami. 1977. Studies on bacteriophage fd DNA. IV: the sequence of messenger RNA for the major coat protein gene. *J. Mol. Biol.* 111:487–507.
222. Sutcliff, J. G. 1978. Nucleotide sequence of the ampicillin resistance gene of *E. coli* plasmid pBR322. *Proc. Natl. Acad. Sci. U. S. A.* 75:3737–3741.
223. Swan, D., H. Aviv, and P. Leder. 1972. Purification and properties of biologically active messenger RNA for a myeloma light chain. *Proc. Natl. Acad. Sci. U. S. A.* 69:1967–1977.
224. Swank, R. T., and T. Paigen. 1973. Biochemical and genetic evidence for a macromolecular β-glucuronidase complex in microsomal membranes. *J. Mol. Biol.* 77:371–389.
225. Szczesna, E., and T. Boime. 1976. mRNA-dependent synthesis of authentic precursor to human placental lactogen: conversion to its mature hormone form in ascites cell-free extracts. *Proc. Natl. Acad. Sci. U. S. A.* 73:1179–1183.
226. Tabas, I., and S. Kornfeld. 1980. Biosynthesis intermediates of β-glucuronidase contain high mannose oligosaccharides with blocked phosphate residues. *J. Biol. Chem.* 255: 6633–6639.
227. Talmadge, K., J. Kaufman, and W. Gilbert. 1980. Bacterial mature preproinsulin to proinsulin. *Proc. Natl. Acad. Sci. U. S. A.* 77:3988–3992.
228. Talmadge, R., S. Stahl, and W. Gilbert. 1980. Eukaryotic signal sequence transport insulin antigen in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 77:3369–3373.
229. Thibodeau, S. N., and K. A. Walsh. 1980. Processing of precursor proteins by preparations of oviduct microsomes. *Ann. N. Y. Acad. Sci.* 343:180–190.
230. Thomas, P. E., A. Y. H. Lu, S. B. West, D. Ryan, G. T. Miwa, and W. Levin. 1977. Accessibility of cytochrome P-450 in microsomal membranes—inhibition of metabolism by antibodies to cytochrome P-450. *Mol. Pharmacol.* 13:819–831.
231. Tomita, M., and V. T. Marchesi. 1975. Amino acid sequence and oligosaccharide attachment sites of human erythrocyte glycophorin. *Proc. Natl. Acad. Sci. U. S. A.* 72: 2964–2968.
232. Urbanski, G. J., and E. Margoliash. 1977. The antigenicity of cytochrome *c*. *In* Immunocytochemistry of Enzymes and Their Antibodies. M. R. J. Salton, editor. John Wiley & Sons, New York. 203–225.
233. Varenne, S., M. Piovant, J. M. Pages, and C. Lazdunski. 1978. Evidence for synthesis of alkaline phosphatase on membrane-bound polysomes in *Escherichia coli*. *Eur. J. Biochem.* 86:603–606.
234. Vassalli, P., R. Tedghi, B. Lisowska-Bernstein, A. Tartakoff, and J.-C. Jaton. 1979. Evidence for hydrophobic region within heavy chains of mouse B lymphocyte membrane-bound IgM. *Proc. Natl. Acad. Sci. U. S. A.* 76:5515–5519.
235. Von Figura, K., and E. Weber. 1978. An alternative hypothesis of cellular transport of lysosomal enzymes in fibroblasts. *Biochem. J.* 176:943–950.
236. Von Heijne, G., and C. Blomberg. 1979. Trans-membrane translocation of proteins. (the direct transfer model). *Eur. J. Biochem.* 97:175–181.
237. Walter, P., R. C. Jackson, M. M. Marcus, V. R. Lingappa, and G. Blobel. 1979. Tryptic dissection and reconstitution of translocation activity for nascent presecretory proteins across microsomal membranes. *Proc. Natl. Acad. Sci. U. S. A.* 76:1795–1799.
238. Warren, G. B., and B. Dobberstein. 1978. Protein transfer across microsomal membranes reassembled from separated membrane components. *Nature (Lond.).* 273:569–571.
239. Weissman, G., G. Sessa, and A. W. Bernheimer. 1966. Staphylococcal alpha-toxin: effects on artificial lipid spherules. *Science (Wash. D. C.).* 154:772–774.
240 Welch, W. J., and B. M. Sefton. 1979. Two small virus-specific polypeptides are produced during infection with Sindbis virus. *J. Virol.* 29:1186–1195.
241. Welch, W. J., and B. M. Sefton. 1980. Characterization of a small nonstructural viral polypeptide present late during infection of BHK cells by Semliki Forest virus. *J. Virol.* 33:230–237.
242. Welton, A. F., and S. D. Aust. 1974. The effects of 3-methylcholanthrene and phenobarbital induction on the structure of the rat liver endoplasmic reticulum. *Biochim. Biophys. Acta.* 373:197–210.
243. Wickner, W. 1979. Assembly of proteins into biological membranes. The membrane trigger hypothesis. *Annu. Rev. Biochem.* 48:23–45.
243a. Wickner, W. 1980. Assembly of proteins into membranes. *Science* (Wash. D. C.). 210: 861–868.
244. Wickner, W., G. Mandel, C. Zwizinski, M. Bates, and T. Killick. 1978. Synthesis of Phage M13 coat protein and its assembly with membranes *in Vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 75:1754–1758.
245. Williams, J. C., and R. M. Bell. 1972. Membrane matrix disruption by melittin. *Biochim. Biophys. Acta.* 288:255–262.
246. Wirth, D. F., F. Katz, B. Small, and H. F. Lodish. 1977. How a single Sindbis virus mRNA directs the synthesis of one soluble protein and two integral membrane glycoproteins. *Cell.* 10:253–263.
247. Wirth, D. F., H. F. Lodish, and P. W. Robbins. 1979. Requirements for the insertion of the Sindbis envelope glycoproteins into the endoplasmic reticulum membrane. *J. Cell Biol.* 81:154–162.
248. Wool, I. G. 1979. The structure and function of eukaryotic ribosomes. *Annu. Rev. Biochem.* 48:719–754.
249. Yamane, K., and S. O. Nathenson. 1976. Biochemical similarity of papain-solubilized $H\text{-}2^d$ alloantigens from tumor cells and from normal cells. *Biochemistry.* 9:4743–4750.
250. Yoshida, A., J. Lieberman, L. Gaidulis, and C. Ewing. 1976. Molecular abnormality of human alpha$_1$-antitrypsin variant (PIZZ) associated with plasma activity deficiency. *Proc. Natl. Acad. Sci. U. S. A.* 73:1324–1328.
251. Zimmerman, R., U. Paluch, and W. Neupert. 1979. Cell-free synthesis of cytochrome *c*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 108:141–146.
252. Zimmerman, R., U. Paluch, M. Sprinzl, and W. Neupert. 1979. Cell-free synthesis of mitochondrial ADP/ATP carrier protein of *Neurospora crassa*. *Eur. J. Biochem.* 99:247–252.